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FINAL REPORT

BIOLOGICAL EFFECTS OF ULTRAVIOLET RADIATION  
ON PLANT GROWTH AND FUNCTION

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Prepared for  
Environmental Protection Agency  
BACER Program  
Washington, D.C. 20460

**United States  
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Agriculture**



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FINAL REPORT

DIFFERENTIAL SENSITIVITY OF TWO CULTIVARS OF  
CUCUMBER (CUCUMIS SATIVUS L.) TO INCREASED  
UV-B IRRADIANCE:

I. DOSE-RESPONSE STUDIES

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## ABSTRACT

Dose-response studies were conducted at Beltsville, Maryland, on two cultivars of cucumber (Cucumis sativus L.) exposed to a UV-B irradiance gradient representing an increase of 40% to 770% in biologically effective UV (BUV) radiation over normal sunlight. Plants were irradiated in a fiberglass greenhouse in April employing FS-40 fluorescent sunlamps filtered with 0.127 mm Mylar (UV-A) or 0.127 mm cellulose acetate (UV A&B). UV treatment was given 6 hours per day (from 1000 to 1600) for 19 days from the time of seeding. Marked differences in UV-B sensitivity were observed between 'Poinsett' (extremely sensitive) and 'Ashley' (slightly sensitive). Increasing the UV-B level induced chlorosis of the leaves, inhibited leaf and shoot growth, and reduced biomass. These effects were especially marked in 'Poinsett'. 'Ashley' plants required approximately twice the level of BUV as 'Poinsett' to exhibit a 20-25% reduction in dry weight or leaf area. Based on linear regression analysis of the 'Poinsett' data, it was estimated that a maximum proposed decrease in stratospheric ozone content of 20% (or a 40% increase in BUV) would cause a 10% reduction in dry matter production with a 15% decrease in leaf area. A 100% increase in BUV or greater was needed to cause pronounced chlorosis of the leaves and a marked reduction in dry matter production. Such increases would be far in excess of the projected BUV levels expected as a result of stratospheric ozone reduction caused by chlorofluoromethane emissions.





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DIFFERENTIAL SENSITIVITY OF TWO CULTIVARS OF CUCUMBER  
(CUCUMIS SATIVUS L.) TO INCREASED UV-B IRRADIANCE:

I. DOSE-RESPONSE STUDIES

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INTRODUCTION

The influence of stratospheric ozone reduction and the attendant increase in solar ultraviolet-B irradiation (UV-B, 280-320 nm) on the biosphere have been of recent concern (Molina and Rowland, 1974).

During the Climatic Impact Assessment Program (CIAP) in 1972-1975, numerous studies were conducted on the response of higher plants to increased UV-B irradiation (Ambler et al., 1975; Basiouny et al., 1978; Biggs, 1975; Biggs and Basiouny, 1975; Brandle et al., 1977; Caldwell, 1977; Krizek, 1975b; Nachtwey, 1975; Sisson and Caldwell, 1976, 1977; Van and Garrard, 1975; and Van et al., 1976). Despite the wealth of

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<sup>3/</sup> Abbreviations: PAR: photosynthetically active radiation (400-700 nm); UV-B: 280-320 nm region; BUV: biologically effective UV irradiance in weighted  $\text{mW m}^{-2}$ ; UV-B SE: UV-B sun equivalent =  $3.06 \text{ weighted mW} \cdot \text{m}^{-2}$  BUV; FS-40 lamps: Westinghouse FS-40 fluorescent sunlamps; M-5: 0.127 mm (0.005 in. = 5 mil) Mylar; CA-5: 0.127 mm (0.005 in. = 5 mil) cellulose acetate.



data collected during this period, relatively little is known about UV-B dose-response relationships for higher plants. Such information is critical in order to assess the biological impact of increased UV-B irradiation caused by stratospheric ozone reduction (Anon., 1977; Krizek, 1975a, 1976, 1977a).

In the course of screening a range of selected species for comparative sensitivity or resistance to broad-band UV-B irradiation, two cultivars of cucumber, 'Poinsett' and 'Ashley', were discovered that differed markedly in their response to increased levels of UV-B irradiation (Krizek, unpublished results, 1976). The present study was conducted to establish dose-response relationships for these cultivars and to determine threshold levels of biologically effective UV (BUV) irradiation required to induce UV-B damage in these two cultivars.

Regression equations are presented to provide a means for assessing the potential biological impact of a projected increase in UV-B irradiance on leaf growth and dry matter production using 'Poinsett' cucumber as a model of a highly sensitive plant.



## MATERIALS AND METHODS

Plant Material. 'Poinsett' and 'Ashley' cucumbers were investigated:

1) because of their differences in UV-B sensitivity observed in an earlier screening program; 2) their rapid growth rate; 3) their uniformity in size; and 4) their prostrate habit for the first 2-3 weeks. Experiments were repeated three times (February, April and June). Data reported here (obtained during April 1-19, 1977) are representative of the dose-response relationships obtained.

Cultural Conditions. Plants were grown for 19 days from seed in 12.5 cm dia. white plastic pots containing a peat-vermiculite mix (Jiffy Mix)<sup>4/</sup>. Five seeds were planted in each pot, using a special template. After 7 days, the seedlings were thinned to one per pot. UV-B irradiation was begun at the time of planting the seed. Minimum night temperatures in the greenhouse did not go below 20 C; day temperatures did not exceed 35 C. Natural daylight and photoperiod were used. The plants were fertilized daily with a 1/4 strength ASHS Hoagland solution (Hammer et al., 1978).

UV Source. In order to provide a gradient in UV-B irradiance, four set-ups were constructed in a fiberglass-covered greenhouse (Table 1). Each set-up contained four fixtures, each containing two FS-40 lamps. One set-up contained eight FS-40 lamps filtered with Mylar (M-5) as a

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UV-B control with the center four lamps maintained at a distance of 1 m and the outer four lamps at a distance of 0.75 m above the plants. Each of the other three set-ups contained eight FS-40 lamps filtered with cellulose acetate (CA-5) with the center four lamps kept at a distance of 1.43, 0.92, or 0.54 m and the outer four lamps kept at a distance of 1.22, 0.70, or 0.54 m respectively above the plants.

The pots were arranged in 8 rows x 12 columns per set-up, each covering an area of  $1.73 \text{ m}^2$  (see Carns et al., 1977). This arrangement was divided into four quadrants (replicates) and the pots were positioned equidistantly from the center of each set-up. Pots of 'Poinsett' and 'Ashley' were alternated at 0.15 m intervals along the x and y axes (corresponding to rows and columns) in order to obtain comparable levels of UV-B irradiance for each cultivar (Table 2).

The experiment was conducted according to a standard protocol described by Krizek (1977b). The lamps were aged 100 hours and the CA filters were aged for 6 hours prior to use. The CA filters were changed twice weekly (because of degradation by the short wave UV). The heights of lamps above canopies were adjusted as the plants grew to maintain the specified levels of UV-B irradiance.

UV Measurements. Broad-band UV-B irradiances were determined at every pot location at the beginning and end of each experiment by means of a broad-band radiometer developed by the Instrumentation Research Laboratory (IRL UV Meter) (Norris, 1977; Rowan and Norris, 1978). Mean values for each of the four set-ups are given in Table 1.

The IRL UV Meter was used to obtain the readings presented in Table 1. The instrument consists of a solar-blind vacuum photo-diode (Hamamatsu R403), an integrated circuit amplifier, and a microammeter





packaged in a meter case (Rowan and Norris, 1978; Norris, 1977). The spectral sensitivity of the IRL Meter in the UV-B region is relatively flat, with maximum sensitivity at 300 nm. The detector is insensitive at wavelengths longer than 400 nm.

Narrow-band UV irradiances were determined at selected pot locations for every nm wavelength from 250 to 369 nm with an automated spectro-radiometer developed by IRL (Norris, 1977; Rowan and Norris, 1978) and commercially available from Optronics Laboratories, Inc., Silver Spring, Md.

BUV weighted irradiances are reported as  $\text{mW}\cdot\text{m}^{-2}$ , the biologically effective UV irradiances derived from the A<sub>2</sub>9 weighting function described by Thimijan et al., 1978 and Carns et al., 1977. Since UV irradiation employed in this study was obtained by filtering FS-40 lamps with CA-5, BUV was essentially confined to the UV-B region. Mean BUV values for each set-up are given in Table 1.

Unweighted spectral irradiances in the UV-B region (Table 1) were obtained by summing measured values at each nanometer from 280-320 nm or using the regression equations developed to estimate the UV-B exposures (Krizek and Koch, 1978). Dividing the  $\text{mW}\cdot\text{m}^{-2}$  of biologically effective UV irradiance by  $3.06 \text{ mW}\cdot\text{m}^{-2}$  [the  $\text{mW}\cdot\text{m}^{-2}$  BUV of one Beltsville control sunshine; i.e., 1 UV-B sun equivalent (SE)] provides the fraction of BUV relative to that of 1 SE (Thimijan et al., 1978).

Harvest and Data Analyses. Plants were harvested after 19 days of UV-B irradiation. An index of injury scale was developed for scoring the extent of leaf chlorosis (Table 2). Leaf areas were then measured with a Lambda LI-COR leaf area meter. Fresh and dry weights of the shoots were taken; the latter were recorded after drying the samples in



a forced draft oven at 80 C for 48 hr. Data on plant height and node number were also taken but are not reported since they showed little or no differences.

Means, standard deviations, standard errors of the mean, and linear and quadratic regressions on weighted BUV were calculated for all parameters. Since quadratic regression of the data yielded no significant improvement of the correlation coefficient (r values), only linear regression data are presented. Data were analyzed separately by cultivar and set-up.

Since the steepest UV gradient was obtained under set-up 4 (Table 1)-i.e., that having a mean BUV level of  $15.3 \text{ mW} \cdot \text{m}^{-2}$  (or 5.0 UV SE)-data obtained for this set-up were used in calculating the regression equations presented.



## RESULTS AND DISCUSSION

Comparative Phytotoxic Effects of UV-B Irradiation. Leaves of 'Poinsett' cucumber plants irradiated for 19 days under CA-5 filtered FS-40 lamps developed marked interveinal and marginal chlorosis with crinkling distortion evident at the tip and along the margins of the leaves (Fig. 1-5). UV damage was observed within 1-2 days after seedling emergence and increased in severity as the leaves expanded and UV-B irradiance increased (Fig. 5). The index of injury under a maximum of 6.7, 11.0, or 15.3  $\text{mW}\cdot\text{m}^{-2}$  BUUV (2.8, 5.4, or 8.7 UV-B SE) was 3, 5, or 9, respectively, (Table 3, Fig. 6). These values represented about 15, 25, or 45% chlorosis, respectively (Table 1). Leaves of 'Ashley' cucumber plants, on the other hand, never reached an index of injury above 3 (15% chlorosis) even when exposed to 15.3  $\text{mW}\cdot\text{m}^{-2}$  BUUV (Table 3). For corresponding mean UV-B doses, 'Ashley' plants exhibited one-third to one-half as much chlorosis as those of the cultivar 'Poinsett' (Table 3).

Under a 40% increase in BUUV or (1.4 UV-B SE) (Table 1) which corresponds to a 20% decrease in  $\text{O}_3$  content in the stratosphere, leaves of 'Poinsett' cucumber plants showed an injury scale of 1 (5% or less chlorosis, while 'Ashley' plants showed an injury scale of 0 (no chlorosis) (Table 3). The negative regression of injury index for the 'Poinsett' cultivar, as measured by leaf chlorosis and weighted BUUV irradiance in  $\text{mW}\cdot\text{m}^{-2}$ , is shown in Fig. 6. The  $r$  value (i.e., 0.81) indicates that the regression equation described may be used to estimate leaf injury expected at a given BUUV irradiance level (Krizek and Koch, 1978). Since no significant regression could be established for leaf injury on BUUV for the cultivar 'Ashley' ( $r = 0.35$ ), the regression is not shown.



The threshold level for chlorosis in 'Poinsett' cucumber leaves varied with season, increasing in the spring and summer and decreasing in the fall and winter, suggesting a difference in photorepair capability with season and amount of PAR.

Influence of UV-B Irradiation on Vegetative Growth. At a mean BUUV level of  $6.73 \text{ mW}\cdot\text{m}^{-2}$  (a 120% increase in BUUV, or 2.2 UV-B SE), (Table 1) and 'Ashley' cucumber plants showed approximately equal (6-10%) reduction in fresh weight of tops (Table 3) and total leaf area (Fig. 7) as compared to the Mylar control plants. Mean dry weight loss, however, at this BUUV level was greater in the case of 'Poinsett' (5.8%) than for 'Ashley' (1.4%) when compared to their Mylar controls (Fig. 8).

UV-B irradiances in excess of  $6.73 \text{ mW}\cdot\text{m}^{-2}$  caused greater reductions in leaf area and dry weight of tops of 'Poinsett' plants than for 'Ashley' plants (Figs. 7, 8). The 'Ashley' cultivar required about twice as much BUUV as 'Poinsett' to produce a 20-25% reduction in leaf area or dry weight (Figs. 7, 8). Leaf size was reduced by increased UV irradiation to a greater extent than was dry weight of tops. At  $11.02 \text{ mW}\cdot\text{m}^{-2}$  BUUV or greater, vegetative growth as measured by fresh weight of tops (Table 3), total leaf area (Fig. 7), and dry weight of tops (Fig. 8), was markedly impaired in both cultivars.

When dry weight data for the 48 'Poinsett' plants under the UV set-up with the widest exposure range-4.9 to  $26.6 \text{ mW}\cdot\text{m}^{-2}$  of BUUV (1.6 to 8.7 UV-B SE) and a mean of  $15.3 \text{ mW}\cdot\text{m}^{-2}$  BUUV (5.0 UV-B SE)-were subjected to linear regression analysis, a correlation coefficient of 0.77 was obtained (Fig. 9). For each unit increase in BUUV, the loss in dry weight would be predicted to be 30.60 mg. On the basis of one SE, a 37% increase in BUUV ( $4.2 \text{ mW}\cdot\text{m}^{-2}$  BUUV or 1.37 UV-B SE) would be expected





to reduce dry weight of 'Poinsett' cucumber plants by 10%;  $10.5 \text{ mW}\cdot\text{m}^{-2}$  BUUV (or 3.43 SE) would reduce it by 25%; and  $21.0 \text{ mW}\cdot\text{m}^{-2}$  BUUV (or 6.86 UV-B SE) to reduce it by 50%. Since actual reductions in dry weight obtained at the higher BUUV levels were less than this prediction and since relatively few data points were collected for UV irradiances in excess of  $15.3 \text{ mW}\cdot\text{m}^{-2}$  BUUV (5.0 UV-B SE), the equation is of greatest value below this point.

Assuming a 20% decrease in stratospheric  $\text{O}_3$  reduction caused by chlorofluoromethanes (CFM's) with a 40% corresponding increase in surface level BUUV [actually likely to be higher than this since the curve is non-linear above 10%  $\text{O}_3$  reduction (IMOS, 1975)], one finds on the basis of this regression curve an approximate 10% decrease in dry weight for a highly sensitive cucumber cultivar such as 'Poinsett'.

Linear regression analysis of the leaf area data for 48 'Poinsett' cucumber means on BUUV resulted in a correlation coefficient of 0.81 (Fig. 10). For each unit increase in BUUV, the decrease in leaf area would be predicted to be  $8.84 \text{ cm}^2$ . On the basis of one SE  $2.8 \text{ mW}\cdot\text{m}^{-2}$  (0.90 UV-B SE) or 90% of present BUUV levels can reduce leaf area in 'Poinsett' cucumber under the conditions of the experiment by 10%;  $6.7 \text{ mW}\cdot\text{m}^{-2}$  BUUV (2.2 UV-B SE), a 120% increase, would be needed to reduce leaf area by 25%;  $13.8 \text{ mW}\cdot\text{m}^{-2}$  BUUV (4.5 UV-B SE), a 350% increase, would be required to reduce leaf area by 50%. Again, assuming a 20% maximum decrease in stratospheric ozone reduction from CFM's, one could predict an approximate 15% decrease in total leaf area for this plant.

The inhibitory effects of high UV-B irradiance on leaf growth in cucumber are consistent with the findings of Sisson and Caldwell (1976, 1977) and Dickson and Caldwell (1978) for Rumex patientia L., previous



work in our laboratory on cotton (Ambler et al., 1975); Alaska pea (Krizek et al., 1975b) and a number of bedding plants (Krizek and Semeniuk, 1975); and early work reviewed by Caldwell (1968, 1971).

Preliminary measurements of stomatal resistance did not indicate a significant difference between Mylar control plants and the green portions of those exposed to increased UV-B, or between 'Ashley' and 'Poinsett' leaves. Moisture content of the tops was slightly, but not significantly, higher in 'Ashley' than 'Poinsett', suggesting that differences in turgor were not responsible for the differences in leaf growth observed (Figs. 8, 10).

Measurements of carbon dioxide exchange rates (CER) made on selected 'Poinsett' cucumber plants at increasing UV levels indicated a significant reduction in CER which was related to the amount of chlorosis observed. UV irradiances in excess of 40% enhancement levels expected to result from CFM-catalyzed destruction of stratospheric ozone content were required to obtain statistically significant differences (Bennett, 1978).

Additional studies are underway to determine the anatomical, physiological, and biochemical bases for the differences in UV-B sensitivity observed between the two cucumber cultivars. Possible explanations to account for these differences might include differences in optical properties of the leaves with different degrees of screening of the responding sites; differences in photoreactivation; differences in biochemical make-up including peroxidase activity; and differences in growth regulator activity.



## CONCLUSIONS

Significant differences in UV-B sensitivity were found between two cucumber cultivars; 'Poinsett' was extremely sensitive and 'Ashley' was slightly sensitive. Evidence was obtained for UV-B induction of: 1) leaf chlorosis; 2) inhibition of leaf growth; and 3) reduction in fresh and dry weight (biomass). These effects were most pronounced under conditions of low PAR and high UV-B irradiation.

Based on regression analysis of plant data obtained under a range of UV irradiances from 4.6 to 26.6 weighted  $\text{mW}\cdot\text{m}^{-2}$  of biologically effective UV (BUV), it was estimated that a maximum decrease in stratospheric  $\text{O}_3$  content of 20% could cause a 10% reduction in dry matter and a 15% decrease in leaf area in the highly sensitive 'Poinsett' cucumber cultivar. Whether reductions in growth of this magnitude could even be detected in nature is questionable.

Increasing the BUV level by at least one SE (i.e., from  $3.06 \text{ mW}\cdot\text{m}^{-2}$  to  $6.12 \text{ mW}\cdot\text{m}^{-2}$  or greater) was required to obtain pronounced chlorosis of the leaves (> 10% chlorosis) with comparable reductions in biomass; these levels would be far in excess, however, of the projected levels of biologically effective UV-B irradiances occurring from CFM-catalyzed reduction of stratospheric ozone.

Further work is needed to elucidate the site and mechanisms of UV-B induced injury in these cucumber cultivars. It is clear that the choice of plant material is a critical factor to the environmental decision-maker in assessing the biological implications of stratospheric ozone reduction and the attendant increase in UV-B irradiation.



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Table 1. Weighted and unweighted UV spectral irradiance under each of four UV set-ups

in the greenhouse. Each set-up contained eight FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in. = 5 mil) Mylar (M-5) or cellulose acetate (CA-5). Data shown are mean values with their standard errors (SE) and ranges of 48 pot locations per cultivar per set-up.

Set-Up	UV Treatment		Cv	IRL UV Meter Reading 10 <sup>3</sup> scale	UV-B SE		BUV <sup>a/</sup> Irradiance Weighted mW·m <sup>-2</sup> Mean ± SE	UV-B Spectral Irradiance Unweighted mW·m <sup>-2</sup>
	Filter	Max Ht. Above Canopy cm			Mean ± SE	Range		
1	M-5	97	'Poinsett' 'Ashley'	3.1 ± 0.1 3.1 ± 0.1	- -	- -	- -	- -
2	CA-5	143	'Poinsett' 'Ashley'	17.6 ± 0.5 17.6 ± 0.5	2.2 ± 0.1 2.2 ± 0.1	1.4-2.8 1.4-2.8	6.73 ± 0.3 6.73 ± 0.3	548.37 548.37
3	CA-5	92	'Poinsett' 'Ashley'	29.6 ± 1.3 29.6 ± 1.3	3.6 ± 0.2 3.6 ± 0.2	1.7-5.4 1.7-5.4	11.02 ± 0.6 11.02 ± 0.6	920.32 920.32
4	CA-5	54	'Poinsett' 'Ashley'	41.0 ± 2.6 40.9 ± 2.6	5.0 ± 0.3 5.0 ± 0.3	1.6-8.7 1.5-8.7	15.30 ± 0.9 15.30 ± 0.9	1273.68 1270.58

<sup>a/</sup> BUV = 3.06 x UV-B SE





Table 2. Criteria for Scoring Chlorosis  
in Cucumber Leaves

Index of Injury	Percent Chlorosis
0	None
0.2	Trace
1.0	5%
2.0	10.0%
3.0	15.0%
4.0	20.0%
5.0	25.0%
6.0	30.0%
7.0	35.0%
8.0	40.0%
9.0	45.0%
10.0	50.0%



Table 3. Influence of increased UV-B irradiation on index of leaf injury, fresh weight and percent dry weight of tops in 'Poinsett' (P) and 'Ashley' (A) cucumber plants irradiated for 19 days in the greenhouse (April 1-20, 1977). Plants exposed to a UV gradient provided by FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in. = 5 mil) Mylar (M-5) or cellulose acetate (CA-5) at various distances above the canopy. Data shown are mean values with their standard errors and ranges of 48 plants of each cultivar per treatment.

Set-Up	UV Treatment		Cv	Index of Injury		Fresh weight		% Dry weight	
	Filter	Max Ht. Above Canopy cm		Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range
1	M-5	97	'Poinsett'	0	0	12.0 $\pm$ 0.3	8.5-17.6	9.2 $\pm$ 0.9	7.5-11.6
			'Ashley'	0	0	12.7 $\pm$ 0.3	8.3-19.3	9.3 $\pm$ 0.1	7.0-10.5
2	CA-5	143	'Poinsett'	1.5 $\pm$ 0.1	1-3	11.1 $\pm$ 0.2	8.4-15.2	9.5 $\pm$ 0.1	8.4-11.0
			'Ashley'	0.7 $\pm$ 0.1	0-3	11.7 $\pm$ 0.2	8.9-14.7	10.0 $\pm$ 0.1	8.9-11.4
3	CA-5	92	'Poinsett'	2.8 $\pm$ 0.2	1-5	9.2 $\pm$ 0.3	4.4-13.2	9.4 $\pm$ 0.1	8.5-11.1
			'Ashley'	0.9 $\pm$ 0.1	0-3	11.3 $\pm$ 0.3	6.7-15.6	10.0 $\pm$ 0.1	9.2-11.3
4	CA-5	54	'Poinsett'	4.5 $\pm$ 0.3	1-9	8.6 $\pm$ 0.4	4.0-13.2	9.5 $\pm$ 0.2	6.3-15.7
			'Ashley'	1.5 $\pm$ 0.2	0-3	10.2 $\pm$ 0.3	6.6-15.6	10.1 $\pm$ 0.1	9.4-11.0



Fig. 1    Appearance of 'Poinsett' (green label) and 'Ashley' (yellow label) cucumber plants grown for 19 days from seeding in the greenhouse under FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) Mylar. Plants received UV-A irradiation but no supplemental UV-B irradiation. Note absence of chlorosis.













Fig. 2 Appearance of 'Poinsett' (green label) and 'Ashley' (yellow label) cucumber plants after 19 days of enhanced UV-B irradiation (6.73 mean weighted  $\text{mW}\cdot\text{m}^{-2}$  of BUV) provided by FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) cellulose acetate.











Fig. 3    Appearance of 'Poinsett' (green label) and 'Ashley' (yellow label) cucumber plants after 19 days of enhanced UV-B irradiation (11.02 mean weighted  $\text{mW}\cdot\text{m}^{-2}$  of BUV) provided by FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) cellulose acetate.













Fig. 4    Appearance of 'Poinsett' (green label) and 'Ashley' (yellow label) cucumber plants after 19 days of enhanced UV-B irradiation (15.30 mean weighted  $\text{mW}\cdot\text{m}^{-2}$  of BUV) provided by FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) cellulose acetate.











Fig. 5 Comparative sensitivity of 'Poinsett' (top row) and 'Ashley' (bottom row) cucumber plants to increased UV-B irradiation. Plants irradiated for 19 days from time of seeding in the greenhouse under FS-40 fluorescent sunlamps filtered with 0.127 mm Mylar (M-5) or 0.127 mm cellulose acetate (CA-5). The latter plants received a mean level of biologically effective UV (BUV) irradiance of 6.73, 11.02, or 15.30 weighted  $\text{mW}\cdot\text{m}^{-2}$ , respectively.









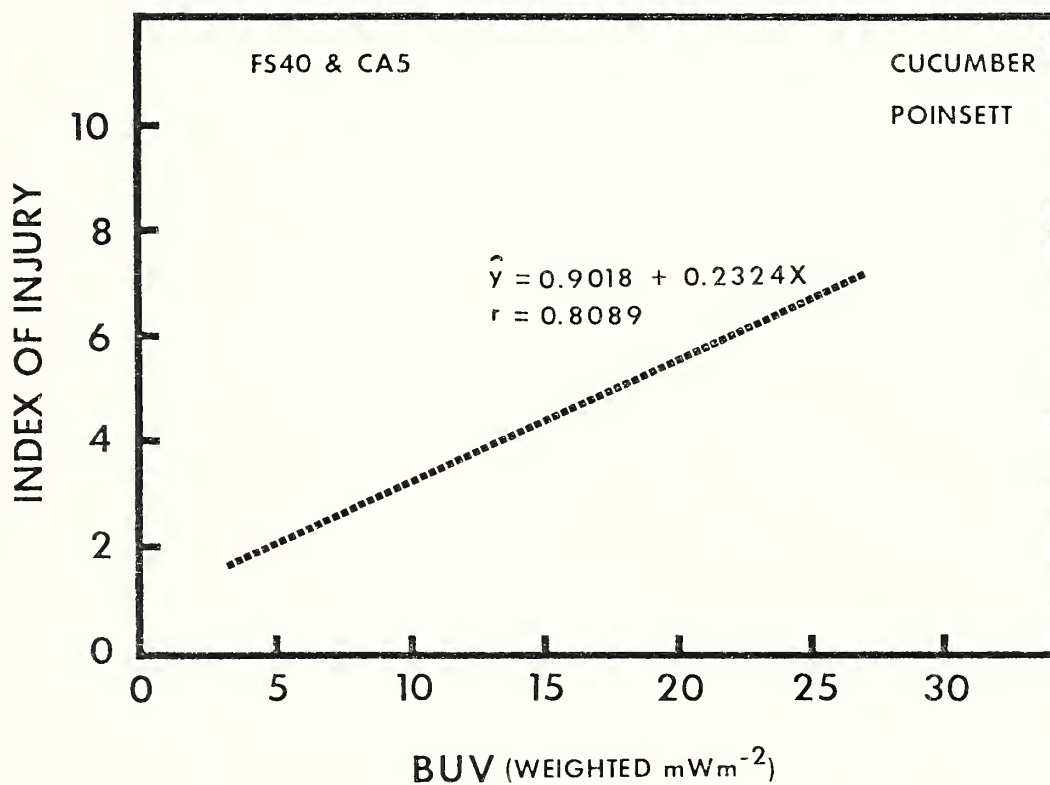


Figure 6. Linear regression of the index of injury for 'Poinsett' cucumber leaves vs. biologically effective UV (BUV) radiation in weighted  $\text{mW}\cdot\text{m}^{-2}$ . Plants were irradiated in the greenhouse for 19 days from seeding under eight FS-40 lamps filtered with 0.127 mm cellulose acetate under a UV gradient ranging from 4.9 to 26.6  $\text{mW}\cdot\text{m}^{-2}$  BUV (1.6 to 8.7 UV-B sun equivalents). (One UV-B sun equivalent = 3.06 weighted  $\text{mW}\cdot\text{m}^{-2}$  of BUV).



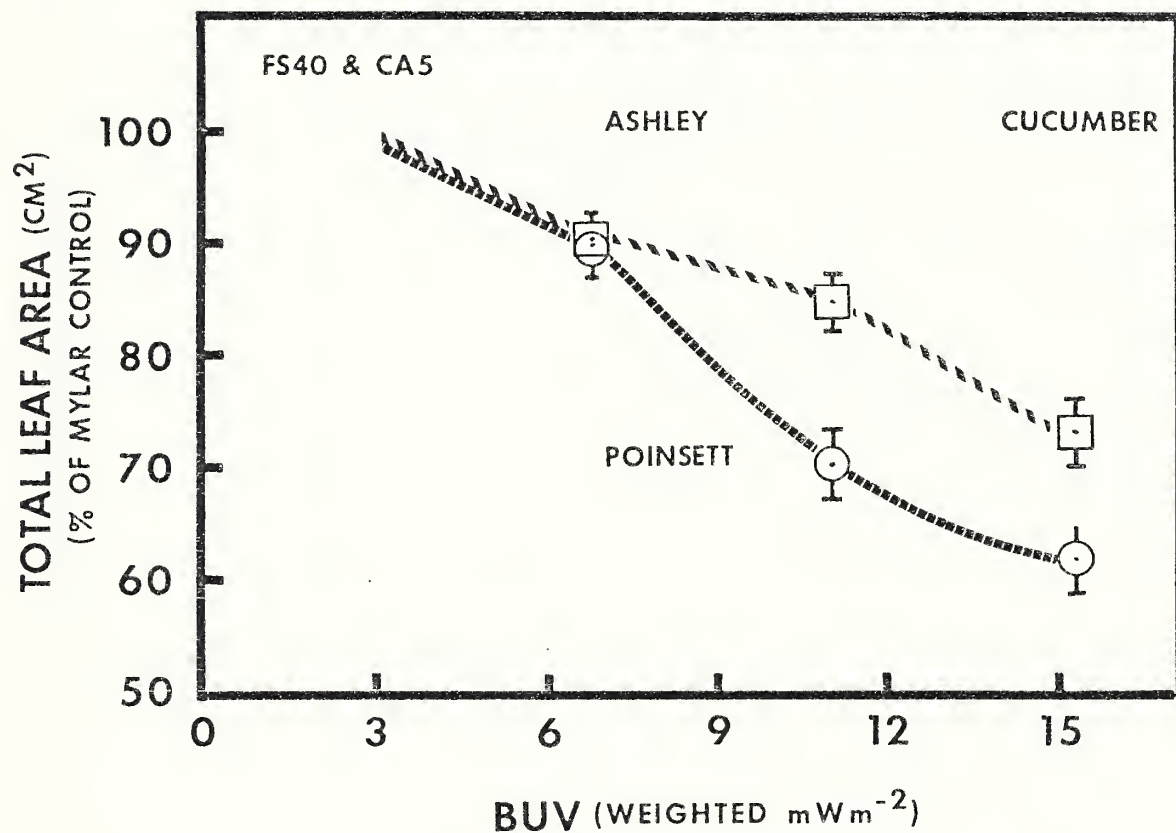


Figure 7. UV dose-response relationship under greenhouse conditions. Comparative leaf areas of 'Poinsett' and 'Ashley' cucumber plants expressed as percentages of Mylar controls. Plants irradiated for 19 days from seeding in the greenhouse under eight FS-40 lamps filtered with 0.127 mm cellulose acetate. Lamps mounted in separate set-ups at 1.43, 0.92, and 0.54 m above the plants. Means and standard errors are shown for 48 plants within each set-up for 6.7, 11.0 or 15.3  $\text{mW}\cdot\text{m}^{-2}$  BUV (2.2, 3.6, or 5.0 UV-B sun equivalents) respectively.



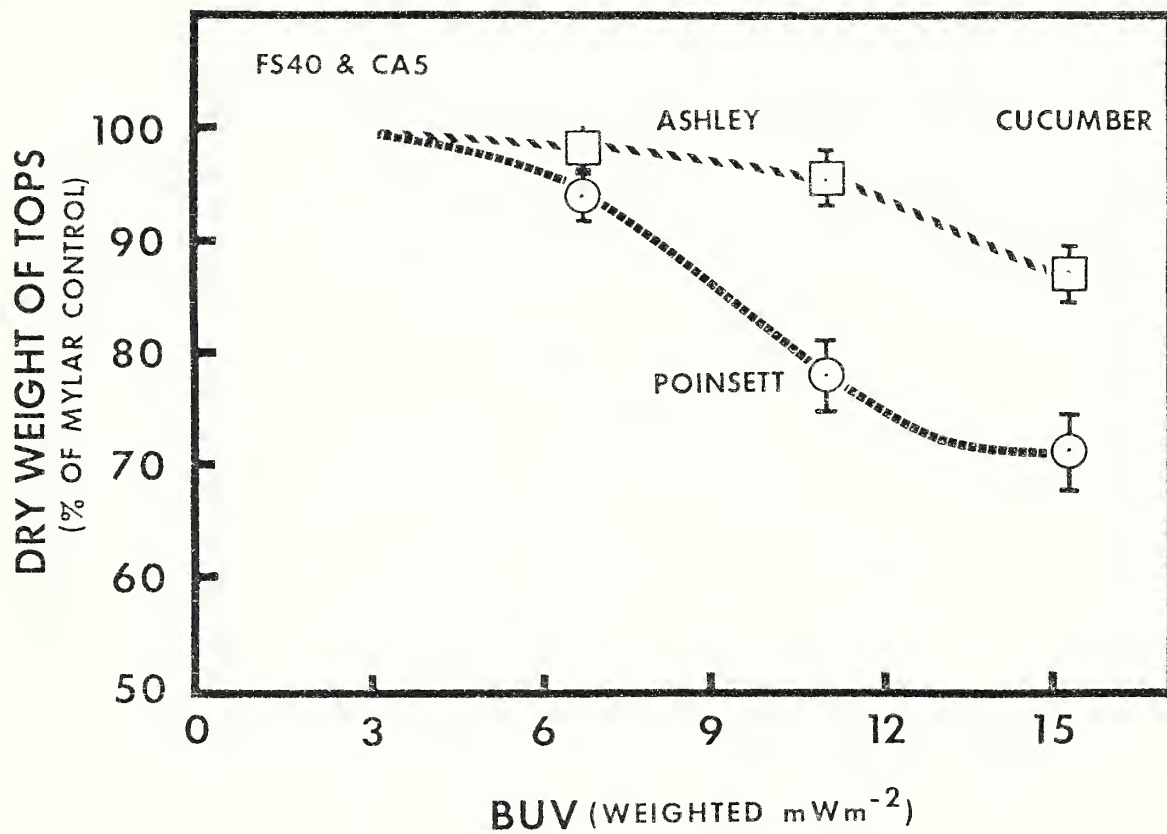


Figure 8. UV dose-response relationship under greenhouse conditions. Comparative dry weights of tops of 'Poinsett' and 'Ashley' cucumber plants expressed as percentages of Mylar controls. Plants exposed to the UV-B irradiation gradients described in Fig. 7.



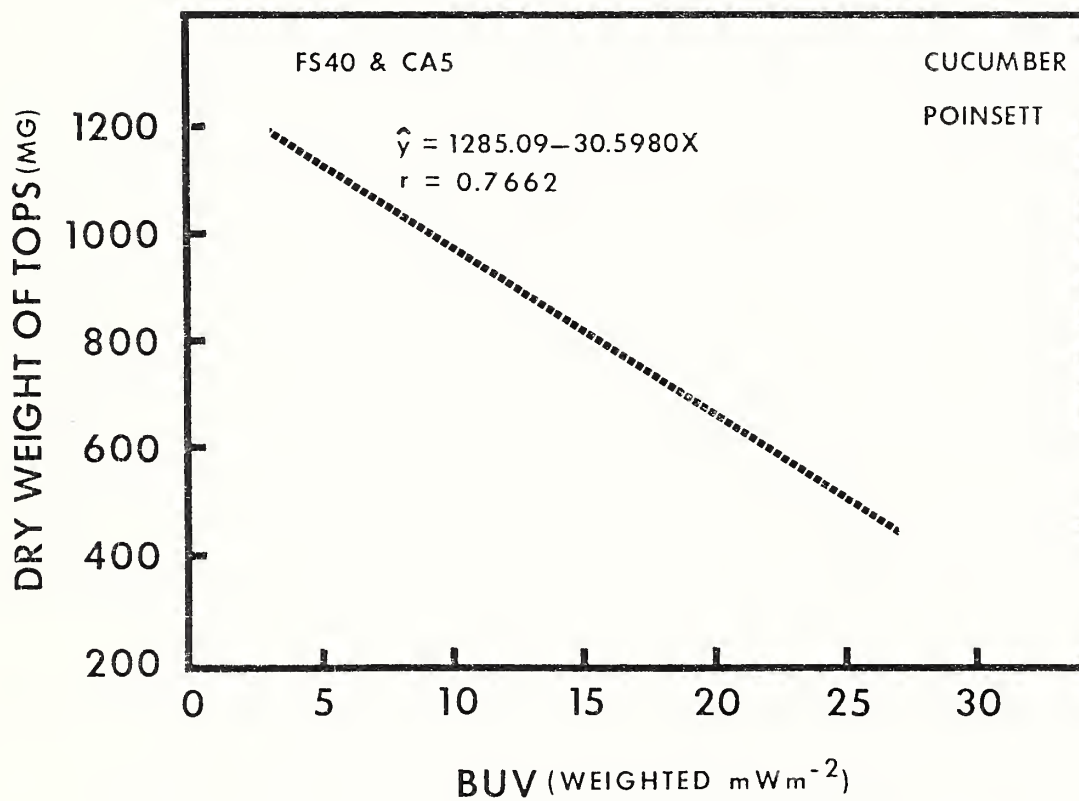


Figure 9. Linear regression of dry weight of tops of 'Poinsett' cucumber plants vs. exposure to biologically effective UV (BUV) radiation in  $\text{mW}\cdot\text{m}^{-2}$ . See Fig. 6 legend.





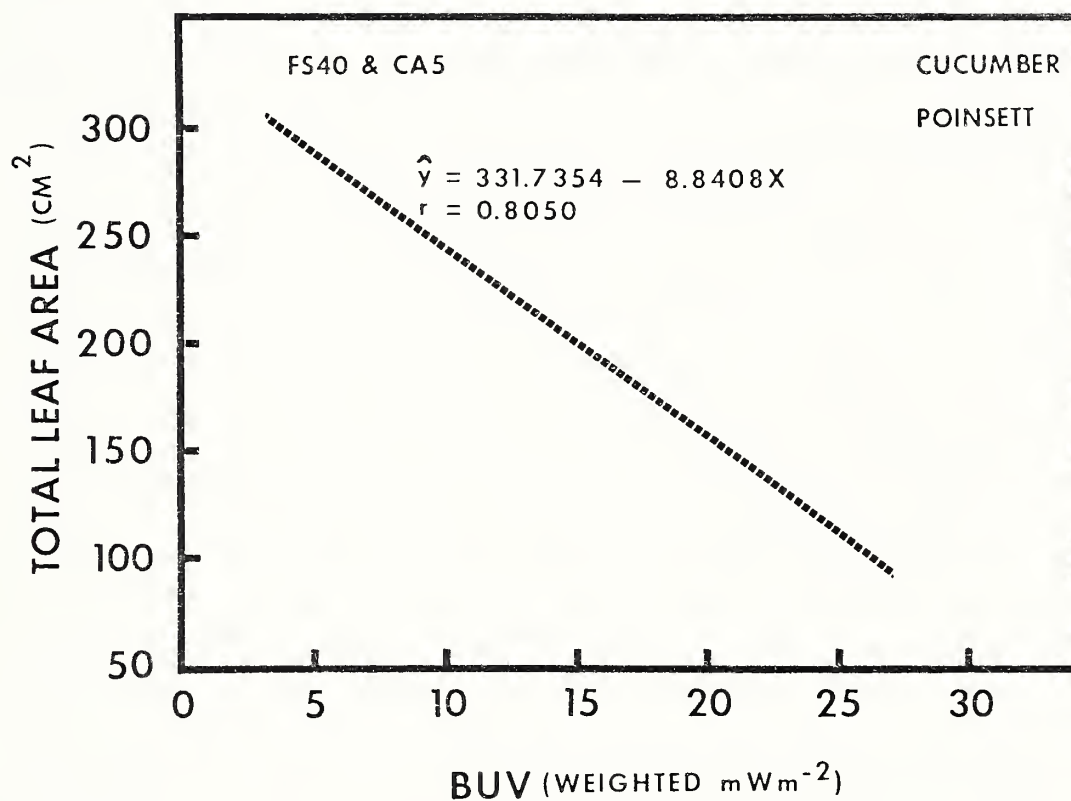


Figure 10. Linear regression of total leaf area of 'Poinsett' cucumber plants vs. biologically effective UV (BUV) radiation in mW·m<sup>-2</sup>. See Fig. 6 legend.







FINAL REPORT

USE OF REGRESSION ANALYSIS IN OBTAINING ESTIMATES  
OF UV SPECTRAL IRRADIANCE UNDER FS-40 FLUORESCENT  
SUNLAMPS FILTERED WITH CELLULOSE ACETATE

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## ABSTRACT

Weighted and unweighted UV spectral measurements were obtained under two Westinghouse FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 inch or 5 mil) cellulose acetate using newly developed broad-band UV radiometers and an automated UV spectroradiometer in 10 cm increments from 20 cm to 110 cm. Correlations were determined between sets of data obtained with the broad-band radiometer and the UV spectroradiometer.

Linear regression analyses were performed on the weighted and unweighted spectral data to obtain regression equations for predicting UV-B irradiance (unweighted  $\text{mW}\cdot\text{m}^{-2}$ ), biologically effective UV (BUV) (weighted  $\text{mW}\cdot\text{m}^{-2}$ ) in the 280-320 nm (UV-B) region, UV-B sun equivalents, and incident UV flux in the UV-B region in  $\text{photons}\cdot\text{m}^{-2} \times 10^{21}$  integrated over a 6-hour exposure.

Examination of the correlation coefficients (r values) indicated excellent agreement between measured and predicted values for all comparisons (r values of 0.9972 to 0.9993).

Use of the regression equations should permit accurate and rapid estimates of both weighted and unweighted UV irradiances at any location in an experimental set-up and provide a useful means of making interlaboratory comparisons of spectral measurements.



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USE OF REGRESSION ANALYSIS IN OBTAINING ESTIMATES OF UV SPECTRAL IRRADIANCE  
UNDER FS-40 FLUORESCENT SUNLAMPS FILTERED WITH CELLULOSE ACETATE

Donald T. Krizek<sup>1/</sup> and E. James Koch<sup>2/</sup>

INTRODUCTION

Broad-band studies on the influence of ultraviolet radiation in the 280-320 nm (UV-B) region on plant growth and development conducted since 1972 during the Climatic Impact Assessment Program (CIAP) have demonstrated the urgent need for improved UV sources and instrumentation (Ambler et al., 1975; Biggs, 1975; Brandle et al., 1977; Krizek, 1975a, b; 1977a, b; Caldwell, 1971, 1972, 1977; Ormrod and Krizek, 1978; Sisson and Caldwell, 1975, 1976, 1977; Skelly et al., 1978; Anon., 1977; Nachtwey, 1975; Van and Garrard, 1975, 1976.

With the recent development of broad-band radiometers and an automated UV spectroradiometer (Norris, 1977; Rowan and Norris, 1978; Carns et al., 1977) and improvements in spectroradiometers used in CIAP (Kostkowski and Saunders, 1977) the researcher now has the means of obtaining greatly improved UV measurements.

The objective of the present study was to provide the investigator involved in the EPA Interagency Biological and Climatic Effects Research (BACER) program a means of obtaining estimates of weighted and unweighted spectral irradiance in the UV-B region. The use of regression analysis of spectral data obtained with both broad-band radiometers and an automated UV spectroradiometer is described.

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A series of regression equations is described for relating weighted and unweighted spectral data in the UV-B region. Use of these equations should facilitate comparison of spectral measurements obtained in different laboratories.



## MATERIALS AND METHODS

### UV Source

UV radiation was provided by Westinghouse<sup>3/</sup> FS-40 fluorescent sunlamps that had been aged at least 100 hours according to a standard protocol (Krizek, 1977b). The lamps were mounted in a single 1.2 meter (4 foot) fluorescent fixture without a special reflector (Thimijan et al., 1978) and covered with 0.127 mm (0.005 in. = 5 mil) cellulose acetate (CA). The CA filters were aged for 6 hours on a specially designed lamp rack before being used in the study (Thimijan et al., 1978).

### UV Instrumentation

Broad-band UV-B irradiance levels were determined by means of three instruments developed by Norris and his associates in the USDA Instrumentation Research Laboratory (IRL) at Beltsville, Maryland, or based on his specifications (Norris, 1977; Rowan and Norris, 1978): (a) an IRL Meter UV-B radiometer (IRL UV meter); (b) an Optronics Laboratories, Inc. Model 725 UV-B radiometer calibrated to read from 0 to 5 UV sun equivalents [or 0 to  $15.3 \text{ mW} \cdot \text{m}^{-2}$  of biologically effective UV (BUV) radiation (Carns et al., 1977)]; and (c) the same instrument calibrated to read from 0 to 10 UV sun equivalents (or 0 to  $30.6 \text{ mW} \cdot \text{m}^{-2}$  of BUV radiation).

The IRL meter was used to obtain unweighted UV-B measurements. The instrument consists of a solar-blind vacuum photo-diode (Hamamatsu R403)<sup>3/</sup>, an integrated circuit amplifier, and a microammeter packaged in a meter case (Rowan and Norris, 1978; Norris, 1977). The circuit provides for four decades

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<sup>3/</sup> Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.





of range switching, referred to as  $10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$ . In the present study, the  $10^7$  and  $10^8$  scales were used. The spectral sensitivity of the IRL meter in the 280 to 320 nm region is relatively flat with maximum sensitivity at 300 nm. The detector is insensitive at wavelengths longer than 400 nm (Rowan and Norris, 1978).

UV spectral irradiances were determined every nm from 250 to 369 nm with an automated spectroradiometer (with a 2 nm bandwidth) developed by IRL and commercially available from Optronics Laboratories, Inc. Specifications for this instrument are described by Norris, 1977, and Rowan and Norris, 1978.

#### UV Measurements

UV measurements were taken with each of these three broad-band radiometers and the automated spectroradiometer described at 10 cm intervals from 10 cm to 110 cm. The sensor was placed under the center of the lamp fixture and adjusted by means of a standard laboratory jack. All measurements were taken in a darkened room with only UV lamps on. Air temperature was maintained at 25°C.

Weighted irradiance levels are reported as  $\text{mW} \cdot \text{m}^{-2}$  UV, the biologically effective UV radiation derived from the weighting function (A<sub>29</sub>) described by Thimijan et al., 1978, and Carns et al., 1977.

Since UV irradiation used in this study was obtained by filtering FS-40 lamps with CA, UV was essentially confined to the UV-B region. Unweighted irradiances in the UV region were obtained by summing measured or calculated values at each nanometer from 250-279 nm (UV-C), 280-320 nm (UV-B), and 321-369 nm (UV-A). Although spectral data were taken previously at every nm from 321-400 nm, no data were taken beyond 369 nm in the present study, since the UV-B portion of the spectrum was the major region of concern in the BACER program.



Dividing the  $\text{mW}\cdot\text{m}^{-2}$  BUV by 3.06 (the  $\text{mW}\cdot\text{m}^{-2}$  BUV of the Beltsville control sunshine) provides the fraction of BUV measured at each location relative to that of one Beltsville control sunshine.

#### Regression Analysis

Linear regression analyses were performed on the weighted and unweighted spectral data to obtain regression equations for predicting UV-B irradiance ( $\text{mW}\cdot\text{m}^{-2}$ ), BUV (weighted  $\text{mW}\cdot\text{m}^{-2}$ ) in the 280-320 nm region, UV-B sun equivalents, and incident UV flux in the UV-B region in  $\text{photons}\cdot\text{m}^{-2} \times 10^{21}$  integrated over a 6-hour day exposure.



## RESULTS AND DISCUSSION

### Weighted and Unweighted Measurements of UV-B Irradiance

Weighted and unweighted spectral measurements obtained at various distances from a pair of FS-40 fluorescent sunlamps filtered with CA are shown in Table 1. In general, there was good agreement between the values obtained for UV-B sun equivalents obtained on the Optronic radiometers and those calculated from unweighted measurements obtained on the IRL UV meter using the conversion factors provided by Thimijan et al. 1978.

The UV-B sun equivalents obtained by summing values every nm from 280-320 nm (Table 1) or 250-329 nm (Table 2) on the automated spectroradiometer by means of computer calculation also agreed within 5 to 10% of those obtained with the broad-band radiometers (Tables 1, 2).

By using a CA filter over the FS-40 sunlamps, little UV radiation in the 250-279 nm region was transmitted (Table 2). The level of BUV radiation transmitted in the 250-279 nm (UV-C); 280-330 nm and 250-330 nm regions under FS-40 lamps filtered with CA are shown in Table 3. Since UV-C radiation contributed virtually no measurable BUV, the total amount of BUV obtained in the 250-330 nm region was approximately the same as that obtained by summing the BUV values in the 280-330 nm region alone (Table 3).

The total unweighted UV irradiance obtained at each 10 nm interval in the 250-279 nm (UV-A), 280-320 nm (UV-B), 321-369 nm (UV-C) and 250-369 nm regions is shown in Table 4. About 50% of the total UV irradiance transmitted in the region of 250-369 nm was at 280-320 nm and 50% at 321-369 nm (Table 4).



### Relationship Between IRL-UV Meter Readings and UV-B Spectral Irradiance

The actual and predicted relationship between broad-band radiometer readings with an IRL UV meter and unweighted spectral irradiance in the 280-320 nm (UV-B) region under two FS-40 sunlamps filtered with CA is shown in Table 5. A plot of these data (Fig. 1) and evaluation of the correlation coefficient ( $r = 0.9995$ ) indicate that the relationship between UV spectral irradiance obtained on the UV spectroradiometer and that predicted by the regression equation from the IRL meter readings agrees very well.

The relationship between IRL UV meter reading and incident UV flux at 280-320 nm measured in  $\text{photons} \cdot \text{m}^{-2} \times 10^{21}$  integrated over a 6-hour period is also shown (Table 5). Since most studies in the BACER program on simulation of ozone depletion were based on 6 hours of UV irradiation per day, the calculations for incident flux are based on this duration of exposure, rather than on the basis of seconds or minutes. The correlation coefficient ( $r$ ) of 0.9976 indicates that there is good agreement between the calculated and predicted incident UV flux in the 280-320 nm region (Table 5). This equation may, therefore, be used to describe the UV-B irradiance in terms used by the photobiologist (see e.g., Seliger, 1978; Rupert, 1978; Rupert and Latarjet, 1978; Caldwell, 1972).

The relationship between IRL meter reading and BUV radiation in the 280-320 nm region is shown in Table 6. The  $r$  values of 0.9992 obtained indicates that the agreement between predicted and actual BUV is nearly perfect. The relationship between IRL meter readings and UV-B sun equivalents is also shown (Table 6). The  $r$  of 0.9991 obtained indicates that there is excellent agreement between predicted and determined UV sun equivalents.





As rule of thumb, instantaneous meter readings on the UV-B broad-band radiometer (IRL Meter) may be converted to UV-B spectral irradiance in photons·m<sup>-2</sup> for a 6-hr exposure by multiplying the reading by 10<sup>21</sup>.

#### Relationship Between UV-B Sun Equivalent and UV-B Spectral Irradiance

The relationship between UV-B sun equivalent as measured with an Optronics Model 725 broad-band radiometer (either the 0-10 scale or the 0-5 scale) and the unweighted UV spectral irradiance in the 238-320 nm region is shown in Table 7. The r's obtained, namely, 0.9996, 0.9995, respectively, indicate that the regression equations described (Table 7) may be used to accurately estimate the total UV-B irradiance obtained at any of the cooperating laboratories participating in the BACER terrestrial effects program that were sent these instruments.

Since all of the Optronics Model 725 radiometers were calibrated by IRL under a pair of FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) CA aged 6 hours, it should be possible to obtain an intra-laboratory and interlaboratory comparison of weighted and unweighted spectral irradiance used at each pot location in any particular study by use of these regression equations provided that no filters were used over the sensor.

The relationship between UV-B sun equivalent as measured with an Optronics Model 725 broad-band radiometer (either the 0-10 scale or the 0-5 scale) and the UV radiation in the 280-320 nm region is shown in Table 8. The r values obtained, namely, 0.9998 and 0.9996, respectively, indicate that the regression equations obtained may be accurately used to estimate the level of UV obtained at any location in the experimental set-up.



The relationship between UV-B sun equivalent as measured with an Optronic Model 725 broad-band radiometer (either the 0-10 scale or the 0-5 scale) and the incident flux in the 280-320 nm region in  $\text{photons} \cdot \text{m}^{-2} \times 10^{21}$  per 6 hr day of UV irradiation is shown in Tables 9 and 10. The  $r$  values obtained (Tables 9 and 10), namely, 0.9972 and 0.9974, respectively, indicate that the regression equations obtained may be accurately used to estimate the incident UV flux in  $\text{photons} \cdot \text{m}^{-2} \times 10^{21}$  received by a plant during a single 6-hr period of UV exposure.

Use of the regression equations described in this report should enable the investigator to make accurate and rapid estimates of both weighted and unweighted UV irradiance at any location in an experimental UV set-up, provided that FS-40 fluorescent sunlamps and 0.127 mm (0.005 in.) cellulose acetate filters are used. By doing so, countless hours can be saved by not having to make spectroradiometric measurements at more than a few selected locations.

Thimijan et al. (1978) have described a method of calculating the spectral power output under both filtered and unfiltered FS-40 lamps as well as Westinghouse BZS lamps by summing values in 5 nm increments from 270 to 320 nm and adding the power output of the Hg lines at 253.6, 289.4, 296.7, 302.2, and 313 nm. This procedure, however, is more time-consuming than the present method of using regression equations.



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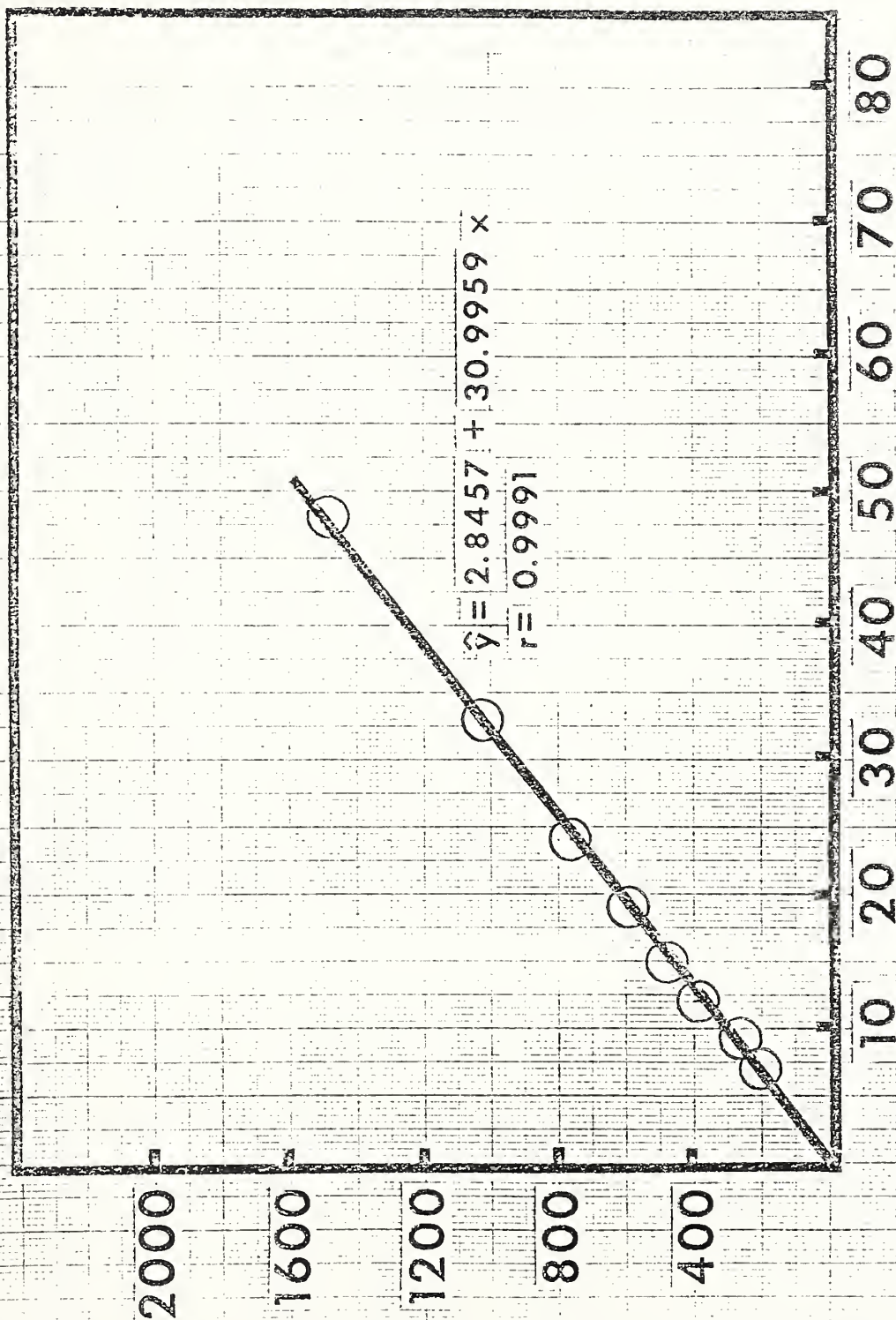
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UV-B IRRADIANCE ( $\text{mW}\cdot\text{m}^{-2}$ )



$$\hat{y} = 2.8457x + 30.9959$$
$$r = 0.9991$$

IRL UV METER READING





Weighted and unweighted measurements for UV-B radiation obtained on November 8, 1977 in the laboratory under two Westinghouse FS-40 fluorescent sunlamps filtered with 0.127 mm cellulose acetate (aged 6 hours) mounted in a single fixture without reflector and room lights off. Detector heads placed under the center of the fixture in a horizontal position.

Distance from sensor (cm)	IRL Meter Reading (10 <sup>7</sup> scale)	UV-B sun Equiv. calculated from IRL meter	UV-B sun equiv. measured on Optronic Radiometer	UV-B sun equiv. measured on spectro-radiometer	BUV mW·m <sup>-2</sup> 280-320nm based on spectro-radiometer	UV-B spectral irradiance (280-320nm) mW·m <sup>-2</sup>
<u>a/</u>	<u>b/</u>	<u>c/</u>	<u>d/</u> (0 to 5 scale)	<u>e/</u> (0 to 10 scale)	<u>f/</u> g/	<u>h/</u>
110	6.90	0.8	0.9	0.8	0.7806	2.3885
100	7.95	0.9	1.1	1.0	0.8861	2.7116
90	9.35	1.1	1.2	1.1	1.0331	3.1613
80	10.0	1.2	1.5	1.4	1.2317	3.7689
70	12.0	1.5	1.7	1.6	1.4758	4.5161
60	15.0	1.9	2.1	2.0	1.7811	5.4503
50	19.0	2.3	2.6	2.5	2.2037	6.7433
40	24.0	3.0	3.3	3.2	2.8514	8.7252
30	33.0	4.1	4.4	4.3	3.7911	11.6008
20	48.0	5.9	off scale	6.1	5.4484	16.6722
						1481.1394

- a/ distance measured from outer wall of lamp to detector head  
b/ measurements at 90, 100, and 110 cm read at 10<sup>8</sup> scale and converted to 10<sup>7</sup> scale  
c/ based on conversion factor provided by R. Thimijar of IRL meter reading x 0.123  
d/ set to a maximum of 5 UV-B sun equivalents, (15.3 mW·m<sup>-2</sup> BUV)  
e/ set to a maximum of 10 UV-B sun equivalents, (30.6 mW·m<sup>-2</sup> BUV)  
f/ weighted portion indicated for 280-320nm region alone  
g/ BUV = biologically effective UV radiation from 280-320nm (weighted mW·m<sup>-2</sup>)  
h/ BUV of 3.06 mW·m<sup>-2</sup> = one UV-B sun equivalent  
actual unweighted flux obtained on an Optronic spectroradiometer; UV-B irradiance measured every nm from 250-369nm but summed from 280-320nm



Table 2.

UV sun equivalents obtained at various distances under two Westinghouse FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) cellulose acetate (aged 6 hrs.). Measurements taken with an Optronic UV spectroradiometer in the laboratory with room lights off.

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Distance from sensor	UV Sun Equiv. 250-279 nm	UV Sun Equiv. 280-329 nm	UV Sun Equiv. 250-329 nm
<hr/>			
110	.0057	0.7806	0.7863
100	.0066	0.8861	0.8927
90	.0075	1.0331	1.0406
80	.0092	1.2317	1.2409
70	.0110	1.4758	1.4868
60	.0136	1.7811	1.7947
50	.0172	2.2037	2.2209
40	.0215	2.8514	2.8729
30	.0288	3.7911	3.8199
20	.0431	5.4484	5.4915

---



Table 3.

Biologically effective UV (BUV) radiation in the 250-279nm, 280-330nm and 250-330nm region ( $\text{mW} \cdot \text{m}^{-2}$ ) under 2 Westinghouse FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) cellulose acetate (aged 6 hrs.). Values based on UV spectroradiometer measurements taken every nm from 250-369nm.

Distance from sensor (cm)	Total BUV 250-279 weighted $\text{mW} \cdot \text{m}^{-2}$	Total BUV 280-330 weighted $\text{mW} \cdot \text{m}^{-2}$	Total BUV 250-330 weighted $\text{mW} \cdot \text{m}^{-2}$
110	.0177	2.3885	2.4062
100	.0202	2.7116	2.7318
90	.0229	3.1613	3.1842
80	.0283	3.7689	3.7972
70	.0335	4.5161	4.5496
60	.0415	5.4503	5.4918
50	.0526	6.7433	6.7959
40	.0659	8.7252	8.7911
30	.0882	11.6008	11.6890
20	.1319	16.6722	16.8041





Table 4.

Total UV irradiance (unweighted  $\text{mW} \cdot \text{m}^{-2}$ ) under two Westinghouse FS-40 fluorescent sunlamps filtered with 0.127 mm (0.005 in.) cellulose acetate (aged 6 hours). Measurements taken with an Optronic UV spectroradiometer every nm from 250-369nm, and total irradiance determined for the UV-A, B, and C regions.

Distance from sensor (cm)	Total Irradiance UV-C 250-279nm $\text{mW} \cdot \text{m}^{-2}$	Total Irradiance UV-B 280-320nm $\text{mW} \cdot \text{m}^{-2}$	Total Irradiance UV-A 321-369nm $\text{mW} \cdot \text{m}^{-2}$	Total Irradiance 250-369nm $\text{mW} \cdot \text{m}^{-2}$
110	0.0223	206.7507	195.8107	402.5837
100	0.0258	234.6552	226.1185	460.7995
90	0.0294	274.8037	263.9468	538.7799
80	0.0361	324.2766	314.7281	639.0408
70	0.0428	392.0929	381.6198	773.7555
60	0.0533	475.1478	467.3057	942.5068
50	0.0668	591.2049	583.7436	1,175.0173
40	0.0850	763.8646	760.5765	1,524.5261
30	0.1127	1,024.9800	1,027.8479	2,052.9406
20	0.1691	1,481.1394	1,489.0918	2,970.4003



Table 5. Actual and predicted relationship between IRL UV meter reading and unweighted UV spectral irradiance in the 280-320 nm (UV-B) region.  $Y_1$  = UV-B irradiance in  $\text{mW}\cdot\text{m}^{-2}$  and  $Y_2$  = incident flux in the 280-320 nm region measured in  $\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  per 6 hour day.

Distance from sensor cm	X	UV-B Irradiance in unweighted $\text{mW}\cdot\text{m}^{-2}$		Incident UV-B flux in in $\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	
		IRL UV Meter Reading ( $10^7$ scale)	Instantaneous Measurement	Error of Prediction	Integrated for 6 hr day
			$Y_1$	$Y_1 - \hat{Y}_1$	$Y_2$
110	6.90		206.7507	-9.9667	6.7459
100	7.95		234.6552	-14.6079	7.6564
90	9.35		274.8037	-17.8537	8.9664
80	10.00		324.2766	11.4719	10.5306
70	12.00		392.0929	17.2964	12.7934
60	15.00		475.1478	7.3636	15.5033
50	19.00		591.2049	-5.5629	19.2901
40	24.00		763.8546	17.1073	24.9237
30	33.00		1024.9800	-7.7304	30.7494
20	48.00		1481.1394	-9.5095	48.3272
					$Y_2 - \hat{Y}_2$
					-.3446
					-.4698
					-.5407
					.4323
					.6723
					.4230
					.2642
					.9658
					-2.0861
					.6957

$$r = 0.995$$

$$X = \text{IRL UV meter reading} \\ (\text{10}^7 \text{ scale})$$

$$\hat{Y}_1 = \text{predicted UV-B irradiance} \\ (\text{mW}\cdot\text{m}^{-2})$$

$$\hat{Y}_1 = 2.8457 + 30.9959X$$

$$r = 0.9976$$

$$X = \text{IRL UV meter reading} \\ (\text{10}^7 \text{ scale})$$

$$\hat{Y}_2 = \text{predicted incident UV-B flux} \\ \text{in } \text{photons}\cdot\text{m}^{-2} \cdot \text{s}^{-1} \text{ per 6 hr day}$$

$$\hat{Y}_2 = 0.2843 + 0.9864X$$



Table 6. Actual and predicted relationship between IRL UV meter reading ( $10^7$  scale) and weighted UV spectral irradiance in the 280-320 nm (UV-B) region.  $Y_1$  = UV-B sun equivalent and  $Y_2$  = biologically effective UV (BUV) radiation in weighted  $mW \cdot m^{-2}$

Distance from sensor cm	UV-B Sun Equivalent			Biologically Effective UV in weighted $mW \cdot m^{-2}$	
	IRL UV Meter Reading ( $10^7$ scale) X	Determined $Y_1$	Error of Prediction $\hat{Y}_1 - Y_1$	Actual $Y_2$	Error of Prediction $\hat{Y}_2 - Y_2$
110	6.90	0.7806	-.0478	2.3885	-.1491
100	7.95	0.8861	-.0624	2.7116	-.1906
90	9.35	1.0331	-.0755	3.1613	-.2272
80	10.00	1.2317	.0487	3.7689	.1547
70	12.00	1.4758	.0640	4.5161	.2073
60	15.00	1.7811	.0261	5.4503	.0996
50	19.00	2.2037	-.0086	6.7433	.0034
40	24.00	2.8514	.0668	8.7252	.2488
30	33.00	3.7911	-.0231	11.6008	-.0013
20	48.00	5.4484	-.0818	16.6722	-.1394

$$r = 0.9991$$

$$X = \text{IRL}_{7\text{UV}} \text{ meter reading } (10^7 \text{ scale})$$

$$\hat{Y}_1 = \text{predicted UV-B sun equivalent}$$

$$\hat{Y}_1 = 0.3900 + 0.1144X$$
  

$$r = 0.9992$$

$$X = \text{IRL}_{7\text{UV}} \text{ meter reading } (10^7 \text{ scale})$$

$$\hat{Y}_2 = \text{predicted BUV (weighted } mW \cdot m^{-2})$$

$$\hat{Y}_2 = 0.1412 + 0.3473X$$



Table 7. Actual and predicted relationship between UV-B sun equivalent as measured with an Optronic Model 725 broad band radiometer (0-10 scale and 0-5 scale) and unweighted UV spectral irradiance ( $\text{mW} \cdot \text{m}^{-2}$ ) in the 280-320 nm (UV-B) region.

Distance from sensor cm	UV-B Sun Equiv. (0-10 scale)		UV-B Irradiance in unweighted $\text{mW} \cdot \text{m}^{-2}$		UV-B Sun Equiv. (0-5 scale)		UV-B Irradiance in unweighted $\text{mW} \cdot \text{m}^{-2}$	
	Measured on Optronic 725 Radiometer $X_1$		Measured on UV Spectro- radiometer $Y_1$	Error of Prediction $\hat{Y}_1 - Y_1$	Measured on Optronic 725 Radiometer $X_2$		Measured on UV Spectro- radiometer $Y_2$	Error of Prediction $\hat{Y}_2 - Y_2$
110	0.8		206.7507	15.2720	0.9		206.7507	10.8111
100	1.0		234.6552	-4.9999	1.1		234.6552	-8.4680
90	1.1		274.8037	11.0603	1.2		274.8037	8.0887
80	1.4		324.2766	-11.7315	1.5		324.2766	-13.2137
70	1.6		392.0929	7.9083	1.7		392.0929	7.4191
60	2.0		475.1478	-5.3898	2.1		475.1478	-3.8931
50	2.5		591.2049	-9.7739	2.6		591.2049	-5.7949
40	3.2		763.8546	-5.7420	3.3		763.8646	1.7224
30	4.3		1024.9800	-9.5873	4.4		1024.9800	3.3283
20	6.1		1481.1394	12.9837	off scale		1481.1394	- -

$r = 0.9996$

$X_1 = \text{UV-B sun equivalent}$

$\hat{Y}_1 = \text{Predicted UV-B irradiance}$

$Y_1 = -1.2273 + 240.8825X$

$r = 0.9995$

$X_2 = \text{UV-B sun equivalent}$

$\hat{Y}_2 = \text{Predicted UV-B irradiance}$

$Y_2 = -16.3863 + 235.9177X$





Table 8. Actual and predicted relationship between UV-B sun equivalent on Optronc Model 725 radiometer (0-10 scale and 0-5 scale) and biologically effective UV (BUV) radiation in the 280-320 nm region.

Distance from sensor cm	UV-B Sun Equiv. (0-10 scale)		Biologically Effective UV in weighted $mW \cdot m^{-2}$		UV-Sun Equiv. (0-5 scale)		Biologically Effective UV in weighted $mW \cdot m^{-2}$	
	Measured on Optronc Radiometer $X_1$	Measured $Y_1$	Error of Prediction $\hat{Y}_1 - Y_1$		Measured on Optronc Radiometer $X_2$	Determined Measured $Y_2$	Error of Prediction $\hat{Y}_2 - Y_2$	
110	0.8	2.3885	.1348		0.9	2.3885	.0976	
100	1.0	2.7116	-.0821		1.1	2.7116	-.1110	
90	1.1	3.1613	.0976		1.2	3.1613	.0728	
80	1.4	3.7639	-.1048		1.5	3.7689	-.1172	
70	1.6	4.5161	.1024		1.7	4.5161	.0983	
60	2.0	5.4503	-.0434		2.1	5.4503	-.0310	
50	2.5	6.7433	-.1005		2.6	6.7433	-.0674	
40	3.2	8.7252	-.0086		3.3	8.7252	.0534	
30	4.3	11.6008	-.1031		4.4	11.6008	.0045	
20	6.1	16.6722	.1082		off scale	16.6722	-	

$r = 0.9998$

$X_1$  = UV-B sun equivalent obtained on Optronc Model 725 radiometer (0 - 10 scale)

$\hat{Y}_1$  = Predicted BUV in the 280-320nm region

$\hat{Y}_1 = .0936 + 2.700X$

$r = 0.9996$

$X_2$  = UV-B sun equivalent obtained on Optronc Model 725 radiometer (0-5 scale)

$\hat{Y}_2$  = Predicted BUV in the 280-320nm region

$\hat{Y}_2 = -0.1020 + 2.6587X$



Table 9. Actual and predicted relationship between UV-B sun equivalent as measured with an Optronics Model 725 broad band radiometer (0-10 scale and 0-5 scale) and incident flux in the 280-320 nm region measured in photons·m<sup>-2</sup>·x10<sup>21</sup> integrated over a 6 hour day.

Distance from sensor cm	UV-B Sun Equiv. Optronics 725 (0-10 scale) X	Total Spectral irradiance 280-320 nm mW·m <sup>-2</sup>	Incident UV-B flux in photons·m <sup>-2</sup> ·x10 <sup>21</sup> per 6 hr day	
			Calculated	Error of Prediction Y - y
110	0.8	206.7507	6.7459	.4523
100	1.0	234.6552	7.6564	-.1697
90	1.1	274.8037	8.9664	.3741
80	1.4	324.2766	10.5806	.3105
70	1.6	392.0929	12.7934	.3699
60	2.0	475.1478	15.5033	.0149
50	2.5	591.2049	19.2901	-.0296
40	3.2	763.8646	24.9237	.2403
30	4.3	1024.9800	30.7494	-2.3626
20	6.1	1481.1394	48.3272	1.4229

$$r = 0.9972$$

X = UV-B sun equivalent

y = predicted incident UV flux at  
280-320 nm in photons·m<sup>-2</sup>  
x10<sup>21</sup> per 6 hour day

$$\hat{y} = 0.1637 + 7.6624X$$



Table 10. Actual and predicted relationship between UV-B equivalent measured with an Optronics Model 725 broad band radiometer (0-5 scale) and incident flux in the 280-320 nm region measured in photons·m<sup>-2</sup> x 10<sup>21</sup> integrated over a 6 hour day.

Distance from sensor cm	UV-Sun Equiv. Optronics 725 (0-5 scale) X	Total Spectral irradiance 280-320 nm mW·m <sup>-2</sup>	Incident UV-B flux in photons·m <sup>-2</sup> x 10 <sup>21</sup> per 6 hr day	
			Calculated	Error of Prediction
110	0.9	206.7507	6.7459	-.0365
100	1.1	234.6552	7.6564	-.3696
90	1.2	274.8037	8.9664	.0485
80	1.5	324.2766	10.5806	-.4728
70	1.7	392.0929	12.7934	.3164
60	2.1	475.1478	15.5033	.1790
50	2.6	591.2049	19.2901	.4066
40	3.3	763.8646	24.9237	1.0574
30	4.4	1024.9800	30.7494	-.9470
20	off scale	1481.1394	48.3272	

$$r = 0.9974$$

X = UV-B sun equivalent

$\hat{y}$  = predicted incident flux at 280-320 nm in photons·m<sup>-2</sup> x 10<sup>21</sup> per 6 hour day

$$\hat{y} = 0.3759 + 7.1183X$$









FINAL REPORT

MULTIPLE EFFECTS OF UV-B IRRADIATION  
ON FUNGAL SPORE GERMINATION

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# ABSTRACT

We investigated the influence of narrow-band UV irradiation in the 265-330 nm region on germination of fungal spores of Cladosporium cucumerinum Ellis & Arth. using a xenon arc lamp and various filters. Based on survival curves and action spectra data, we propose that there are two active regions between 280 and 320 nm (UV-B) that might be influenced by changes in the stratospheric ozone layer: a short-wave portion (265-295 nm) and a long-wave portion (300-330 nm). Action spectrum data obtained with narrow-band interference filters confirmed previous reports of damage to DNA from UV irradiation at 265-295 nm UV and in addition demonstrated significant inhibitory effects of UV irradiation at 300-320 nm. Further studies made of the 300-330 nm portion of the spectrum using a combination of plastic and glass filters showed that the influence of UV irradiation in this region was primarily to produce a non-photoreactivable delay in germ tube outgrowth. The implications of these findings are discussed in relation to the possible impact of stratospheric ozone reduction.





# TABLE

Table 1. Global downward energy fluence in  $\text{J}\cdot\text{m}^{-2}\cdot 5\text{ nm}^{-1}$  for a 6-hr period (assuming an average zenith angle of  $30^\circ$ ) at three ozone concentrations at standard temperature and pressure.





## FIGURES





1. Accumulated percent outgrowth of germ tubes of Cladosporium cucumerinum fungal spores following inhibition and placement on a water agar plate as described in the Materials and Methods. The two symbols are replicate plates, and the vertical lines are one standard deviation. The curve was drawn by inspection.
2. A, B, and C: Survival curves for 5 nm half-band width UV exposures of Cladosporium cucumerinum fungal spores at the indicated central wavelengths. Percent survival was determined at 22 hours and is the percent of control survival. The fluences were obtained by varying the time.
3. Action spectra for UV inhibition of fungal spore germination in Cladosporium cucumerinum plotted from data of Figure 2 (A, B, and C). Action is expressed as reciprocal of the  $\text{joules} \cdot \text{m}^{-2}$  that gave 90% (o) and 37% (●) survival. The vertical lines are the 95% confidence limits.
4. Percent survival of Cladosporium cucumerinum fungal spores following exposure to UV irradiation at 265 nm given at  $0.1 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  (o) and  $1.0 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  (●). Counts were made as in Fig. 2.
5. Percent survival of Cladosporium cucumerinum fungal spores following exposure to a broad band UV source centering on 325 nm (inset) given at  $80 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  (o) and  $800 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  (●). Counts were made as in Fig. 2.
6. Survival curve for exposure of Cladosporium cucumerinum fungal spores to a broad band UV source centering on 330 nm (inset) given at  $400 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ . Counts were made at 22 hours as in Fig. 2.
7. Accumulated percent outgrowth of Cladosporium cucumerinum fungal spores vs duration of exposure to broad band UV irradiation centering



at 330 nm. The indicated values (360, 480, and 600) are  $\text{kJ}\cdot\text{m}^{-2}$  obtained by duration of exposure to  $400 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  for 15, 20, and 25 min., respectively. Control spores received no UV irradiation.

8. Time course for outgrowth of Cladosporium cucumerinum fungal spores showing comparative influence of short-wave and long-wave UV.

A. 275 nm narrow-band UV (5 min.  $0.57 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ; , ).

B. 330 nm broad-band UV (5 min.  $520 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ; , ). Controls (no UV exposure: , ). Plates were incubated in dark (closed symbols) or in light (open symbols).

9. Influence of stratospheric ozone reduction on percent germ tube outgrowth of Cladosporium cucumerinum fungal spores in the UV-B region. Values were calculated from the data of Fig. 2 and Table 2. The values assigned to the curves represent "normal" ozone concentration  $0.32 \text{ atm}\cdot\text{cm}$ ; 12% reduction,  $0.28 \text{ atm}\cdot\text{cm}$ ; and 50% reduction,  $0.16 \text{ atm}\cdot\text{cm}$ .



#### ACKNOWLEDGMENTS

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# Multiple Effects of UV-B Irradiation on Fungal Spore Germination

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## INTRODUCTION

The possibility of stratospheric ozone reduction has increased concern about the potentially harmful effects of irradiation in the spectral region where ozone absorption normally cuts off the sun's ultraviolet radiation. This region (UV-B) is defined as the 280-320 nm region.

The lethal and mutagenic effects of UV absorption at the shorter wavelengths, 250-280 nm (UV-C), are well-documented and are associated with DNA and protein absorption. Previous studies on the effects of UV irradiation showed that the 320-400 nm (UV-A) region acts on a variety of metabolites to produce lethal, inhibitory, and delaying effects (1, 2, 3, 6, 7, 15). Between these two active regions is the UV-B region where there are few major absorption peaks. Because any effect observed at the peak of absorption of a photo-chemically active compound would be observed also in the region of lesser absorption, it seems reasonable to expect that observed effects of UV-A and UV-C irradiation would overlap in the UV-B region.

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Another major characteristic of the UV-B region is the 5-decade decrease in the sun's irradiance from 320-280 nm caused by the sharply increasing absorption coefficient of ozone. It is evident that a reduction in stratospheric ozone concentration could shift this sharp cut-off to shorter wave-lengths, and increase UV irradiance on the short wave side of the cut-off region.

In order to assess the biological effects of this increase in UV irradiance, we need to estimate the effects at those wavelengths where changes in the ozone concentration will have a major effect on the sun's fluence at the earth's surface. In this report we show that for germination of non-proliferating fungal spores (conidia), the action of UV irradiance from 265-295 nm on survival is closely correlated with nucleic acid absorption. From 300-330 nm the response resembles that reported for the near UV region (8, 9) and presumably involves a delay in protein synthesis.

#### MATERIALS AND METHODS

UV Source: The UV source (Schoeffel)<sup>3/</sup> was a 2.5 kW high-pressure xenon arc lamp equipped with collimating lenses, a 15 cm circulating cooled water filter and a mirror at a 45° angle to produce a vertical beam. To provide narrow band irradiation we placed various filters in the parallel beam: The first filter was a 1 cm deep, 1:1 mixture of saturated solutions of  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  and  $\text{CSO}_4 \cdot 7\text{H}_2\text{O}$  in an open dish with parallel sides and a flat quartz bottom. In later experiments the mixture was diluted

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<sup>3/</sup>

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further with distilled water that had no detectable effect on the transmission characteristics. The dish was kept below 50°C with a cooling coil around the outside. The second filter was either a 5 nm half-band width interference filter (Corion) of the selected central wavelength or a combination of plastic and glass filters for the broader bands: for 325 nm, 2 mm pyrex and 0.13 mm cellulose acetate; for 330 nm Corning Nos. 0-54 and 7-54.

The beam was directed from the mirror through the  $\text{NiSO}_4\text{-CoSO}_4$  solution, then through the secondary filter onto a 15 rpm turntable containing the test organisms as described below. The UV irradiance was measured with a pyroelectric radiometer (Molelectron) that could be moved into the beam as needed. From just below the mirror the beam was surrounded by a black box maintained at 21-23°C. A tube surrounded the beam below the filters, and baffles prevented stray radiation from falling on the radiometer or the test organisms.

Experimental Material: The test organisms were conidiospores of the fungus, Cladosporium cucumerinum Ellis & Arth., a leaf pathogen of cucumbers. We used this species because of its ease in cultivation and handling. Its spores normally give 95-98% germination and under natural conditions are exposed in the imbibed state (from dew) to the sun's irradiance during their germination period on the leaf surface.

Cultures of the fungus were maintained on potato-dextrose agar. Spores were harvested from 6-day-old colonies by addition of 10 ml of distilled water to the colony and agitated to obtain a uniform suspension. From the suspension 30 µl was dropped onto the center of a water agar plate. The



water was absorbed by the agar in about 20 minutes leaving an area of imbibed spores, with a diameter of about 1 cm, in an even layer on the agar surface. The spore area was one-third the diameter of the shielded, collimated beam from the xenon arc. Following absorption of the water the covered plates were held in darkness at 40°C for 15-30 minutes until the start of the experimental period.

For exposure, the agar plate without a top was placed in the beam on the turntable and exposed for a period of time that gave the specified photon fluence as determined from the power readings with the radiometer. Uncovered matched control plates were placed in the same box but away from the beam and shielded by mylar. For longer exposure times, viz. 1-6 hours, covered control plates were also included in the box. There were no significant differences in percent outgrowth between covered and uncovered controls.

Following exposure, the plates were covered and incubated in darkness (unless otherwise specified) at 22°C. Germination of spores results in the outgrowth of a germ tube. The presence of the germ tube was used as the indication of survival (percent survival or percent outgrowth).

Observations of germ tube outgrowth were made at 150 x magnification in a darkened room, and when necessary the microscope light source was filtered through red and yellow colored plastic to prevent photoreactivating light from reaching the spores. For each datum point all spores (about 100) in each of five random areas (each 0.56 mm<sup>2</sup>) were counted and the proportion with a distinct germ tube was recorded. Data were expressed as average percent of spores with germ tube outgrowth  $\pm$  the standard deviation. In most cases the outgrowth produced on the exposed plates was expressed as percent of the outgrowth of the control plates. For time course experiments, the plates were removed at intervals for counts, then returned to the incubator.







The method used for spore handling had several advantages: (1) the spores were located on a flat surface minimizing scattering errors; (2) microscopic examination allowed for elimination from the counts of overlapping and clustered spores; and (3) no additional nutrients were necessary for germ tube outgrowth.

## RESULTS

Growth of the Spores: The population of fungal spores on the agar plate produced germ tubes over a period of time. Figure 1 shows the accumulated percent of spores with germ tubes for replicate control plates determined at intervals over a period of 25 hours. Outgrowth began after about 4 hours and reached a maximum after 20 hours. The rate of accumulated outgrowth increased and reached a maximum at about 9 hours, when 55-65% of the spores had germ tubes, and then decreased. The accumulated percent outgrowth superficially resembled a cumulative normal frequency distribution but deviated significantly from normality in most cases.

Survival Curves: Figure 2 (A, B, and C) shows the influence of UV irradiance of the indicated 5 nm bandwidths on outgrowth of germ tubes of Cladosporium cucumerinum fungal spores. Percent of control outgrowth was determined after 22 hours of incubation as described under Materials and Methods. The dose, expressed as  $\text{joules} \cdot \text{m}^{-2}$ , was obtained by varying the time of exposure. The curves for 265-295 nm demonstrate the lack of an exponential relationship between percent outgrowth and dose at low doses. The presence of the shoulder can be attributed to an active dark repair mechanism. The curves for 300-320 nm show only a shallow linear relationship indicating that those wavelengths were relatively ineffective.



Because we are primarily concerned with the effects of solar irradiation, we have concerned ourselves with the low dose parts of the curves, located on the shoulders, corresponding to the sun's irradiance at each wavelength examined.

Demonstration of measurable decreases in germ tube outgrowth at 310-320 nm required long exposure times of up to 6 hours. We could not show the lower part of the survival curves with the use of the narrow band UV filters.

Action Spectrum: Figure 3 shows two action spectra constructed from the data in Figure 2 (A, B, and C). Action is expressed as the reciprocal of the joules·m<sup>-2</sup>. The upper curve shows the joules permitting 90% survival which is on the shoulder of the survival curves. We assumed that the survival curves were nearly linear to 70% and calculated a value for 90% survival from the regression equations developed from the individual points. This calculation introduced a small error and enlarged the 95% confidence interval (vertical lines) but allowed us to make a single action spectrum throughout the 265-320 nm region. The lower curve of Figure 3 shows an action spectrum constructed from the 1/e values (37% survival) from those survival curves that could be extended to 37% survival. These values were obtained from curves drawn by inspection. The similarity of the two curves in the 265-295 nm region (the short-wave portion) indicates that the slope of the shoulder was proportional to the final slope and suggests that the rate of dark repair was nearly proportional to the rate of damage. The 265-295 nm action spectra closely match published spectra (13,16) and can be attributed to damage to DNA.

Values on the 300-320 action spectrum are too high in relation to 265 nm to be attributable to DNA damage. We conclude from these results that there are two active portions in the UV-B region: a short wave portion



(265-295 nm) whose action corresponds to DNA absorption and a long-wave portion (300-330 nm) whose action corresponds to an unknown component(s).

Reciprocity: Figure 4 shows the effect of 265 nm irradiation at two fluence rates:  $0.1$  and  $1.0 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ . For the low rate a neutral density filter was used and the duration of exposure was increased 10-fold. The figure shows that at the low fluence rate the UV irradiation was less effective than at the high rate, indicating a greater efficiency of repair. The ratio of the fluences at the low and high rates in producing a similar percent survival was about 1.9. These data indicate that reciprocity was incomplete.

We could not measure reciprocity at 300-320 nm using the narrow band interference filters because of the long exposures that would be required at low irradiances. Therefore we used a broad band filter, centering on 325 nm, described under Materials and Methods, to provide a higher fluence rate in this spectral region. Figure 5 shows the influence of low ( $80 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) vs high ( $800 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) fluence rates on spore survival. The inset shows the transmission character of the filter determined spectrophotometrically. Reciprocity was nearly complete, indicating that in the 325 nm portion of the spectrum there was no detectable simultaneous repair mechanisms as that shown for the 265 nm band.

Character of 330 nm Inhibition: In the 325 nm experiment described above, the filters used allowed significant transmission below 300 nm. We, therefore, used another filter combination (see Materials and Methods), centering on 330 nm that gave <1% transmission at 300 nm and above 370 nm (inset, Fig. 6).

Figure 6 is a survival curve obtained with the 330 nm broad band filter. As for exposures at 265 nm, the points represent counts at 22 hours after





exposure. We noticed, however, that several hours later additional spores had germinated--an effect not observed at any of the other wavelengths.

The influence of 330 irradiation ( $400 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) on percent outgrowth was determined at intervals over a period of time for control and irradiated spores (Fig. 7). Three fluences were chosen to coincide, respectively, with three points on the survival curve shown in Fig. 6: (1) on the shoulder ( $360 \text{ kJ}\cdot\text{m}^{-2}$ ); (2) where the curve bends ( $480 \text{ kJ}\cdot\text{m}^{-2}$ ); and (3) on the steep part of the curve ( $600 \text{ kJ}\cdot\text{m}^{-2}$ ). The figure shows that broad band UV irradiation centered at 330 nm delayed the start and depressed the rate of outgrowth. Thus, the data in Fig. 7 indicate that the "survival curve" of Fig. 6 is an indication of both a delayed and a depressed rate of outgrowth; the shape of the curve depended on the time at which spores were counted.

Delay, Outgrowth Rate, and Photoreactivation: We compared the effect of narrow-band irradiation at 275 nm (representing the short-wave portion), to the effect of broad-band 330 nm irradiation, (representing the long-wave portion). Both exposures were for 5 min. The fluence rates were  $0.57 \text{ J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and  $520 \text{ J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively. Both fluences were on the shoulders of the percent survival curves. Following exposure, replicate plates were incubated in darkness or under a bank of 1500 mA cool white fluorescent lamps filtered with 0.127 mm (0.005 in) Mylar (short-wave UV cut-off, 3% T at 320 nm). Controls received no UV exposure. Percent outgrowth, shown in Fig. 8, was determined at the intervals indicated in Fig. 7.

Figure 8 shows that the incubation light inhibited the rate of outgrowth of the controls, but did not delay onset of outgrowth. The fluorescent lamps used for incubation contained, in addition to the visible radiation, UV irradiation at 365 nm ( $95 \text{ mJ}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) and at 334 nm ( $9 \text{ mJ}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ).





This irradiation may have produced a cumulative inhibitory effect during the incubation period. Figure 8B shows that exposure to broad band 330 nm followed by either dark or light incubation significantly delayed onset of outgrowth in comparison with the controls. After the delay the rates of outgrowth in dark equalled those in the light. Delay in outgrowth of spores, incubated in either light or dark, was greater from the 275- (Fig. 8A) than from the 330-nm (Fig. 8B) treatment. With spores treated at 330 nm, light during incubation shortened the delay and increased the rate of outgrowth to a value equal to that for the light-incubated control. The results of this experiment and previous ones showed that the effects of the short-wave and long-wave portions of the UV region studied were similar in that both delayed the start of outgrowth of spores. Effects differed between the long-wave and short-wave portions. With the long-wave portion the delay in the start of outgrowth was not affected by light incubation and UV irradiation had little effect on the rate of outgrowth except at high doses (Fig. 7.).

Germ Tube Outgrowth in Relation to Sunshine and Ozone Reduction: By use of the regression equations developed from data in Fig. 2, we determined the effectiveness of each 5 nm wavelength band of solar irradiation in inhibiting germ tube outgrowth. The data of Shettle et al. (11) for solar irradiance were used as a basis for the calculations. We used values for downward global flux,  $30^{\circ}$  zenith angle and, ozone concentrations of 0.32 atm·cm, 0.28 atm·cm, 0.16 atm·cm. Assuming that the fungal spores would be exposed to a maximum of 6 hours of sun in June at the Washington, D.C. latitude, average zenith angle  $30^{\circ}$ , we converted the data to  $\text{J}\cdot\text{m}^{-2}\cdot 5\text{ nm}^{-1}$  for 6 hours. These values are shown in Table 1. Figure 9 shows the calculated percent inhibition that would be expected for the irradiance values at the three ozone concentrations of Table 1. The inhibition assumes no photoreactivation and does not distinguish.



between lethality and temporary delay of spore outgrowth. At 0.32 atm·cm, an approximation of "normal" ozone, i.e. no reduction, the sun's irradiance throughout the 300-320 nm region is sufficient to produce a 5% inhibition at each wavelength, a result also noted by Jagger (5) for the near UV region. A reduction in stratospheric ozone concentration to 0.28 atm·cm would not significantly inhibit germ tube outgrowth, but a reduction to 0.16 atm·cm, a 50% depletion would produce a significant inhibition of nearly 20% at 295-300 nm. The inhibition at 295 nm was somewhat under-estimated because the fluence value was slightly beyond the survival curve shoulder (Fig. 2, B). The effectiveness of the 295-300 nm region in inhibiting germ tube outgrowth of Cladosporium spores agrees with the analysis of Setlow (10) and approximates that of Elkind et al. (2) for effectiveness of normal sunlight in skin cancer production.

#### DISCUSSION

The fungal spore produces the germ tube after a period of germination that is primarily a period of protein synthesis. In our study we used lack of appearance of the germ tube as a measure of damage to the protein-synthesizing system of the spore. Most fungal spores have a complete system for protein synthesis in which enzymes increase in activity and amount during the termination period (12). Protein synthesis and germ tube outgrowth are inhibited by cycloheximide indicating involvement of a ribosomal system. New RNA synthesis occurs, but the extent of the requirement for new RNA has not been established. DNA replication does not occur until the time of outgrowth or after (4,14), but our results indicate that DNA must function at some stage of the germination sequence.



We propose that there are two active regions between 280 and 320 nm. Our results suggest that the short-wave portion primarily affects DNA and the long-wave portion is primarily related to effects of near UV noted by other investigators. Because the DNA-absorbing part indicates the presence of both a dark repair mechanism and photoreactivation, a moderately increased irradiance from the sun might be ineffective in producing an immediate inhibition. Enhanced UV irradiance from the sun, however, might retard the rate of growth, observable even at normal sunlight, that would not be repaired in either darkness or light. We have shown that the critical region for major effects, whether lethal or delaying, is at 295-300 nm, a region that should be thoroughly analyzed.



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Table 1. Global downward energy fluence in  $\text{J}\cdot\text{m}^{-2}\cdot 5\text{nm}^{-1}$  for a 6-hour period (assuming an average zenith angle of  $30^\circ$ ) at three ozone concentrations at standard temperature and pressure. Values calculated from Shettle et al. (1975).

Wavelength 5 nm band	Ozone Concentration		
	0.32 atm·cm	0.28 atm·cm	0.16 atm·cm
320	$3.44 \times 10^4$	$3.59 \times 10^4$	$4.07 \times 10^4$
310	$1.19 \times 10^4$	$1.37 \times 10^4$	$2.10 \times 10^4$
300	$5.62 \times 10^2$	$9.08 \times 10^2$	$3.81 \times 10^3$
295	$1.76 \times 10^1$	$4.22 \times 10^1$	$5.92 \times 10^2$
290	$3.31 \times 10^{-2}$	$1.67 \times 10^{-1}$	$2.21 \times 10^1$
280	$2.27 \times 10^{-16}$	$5.99 \times 10^{-14}$	$1.09 \times 10^{-6}$



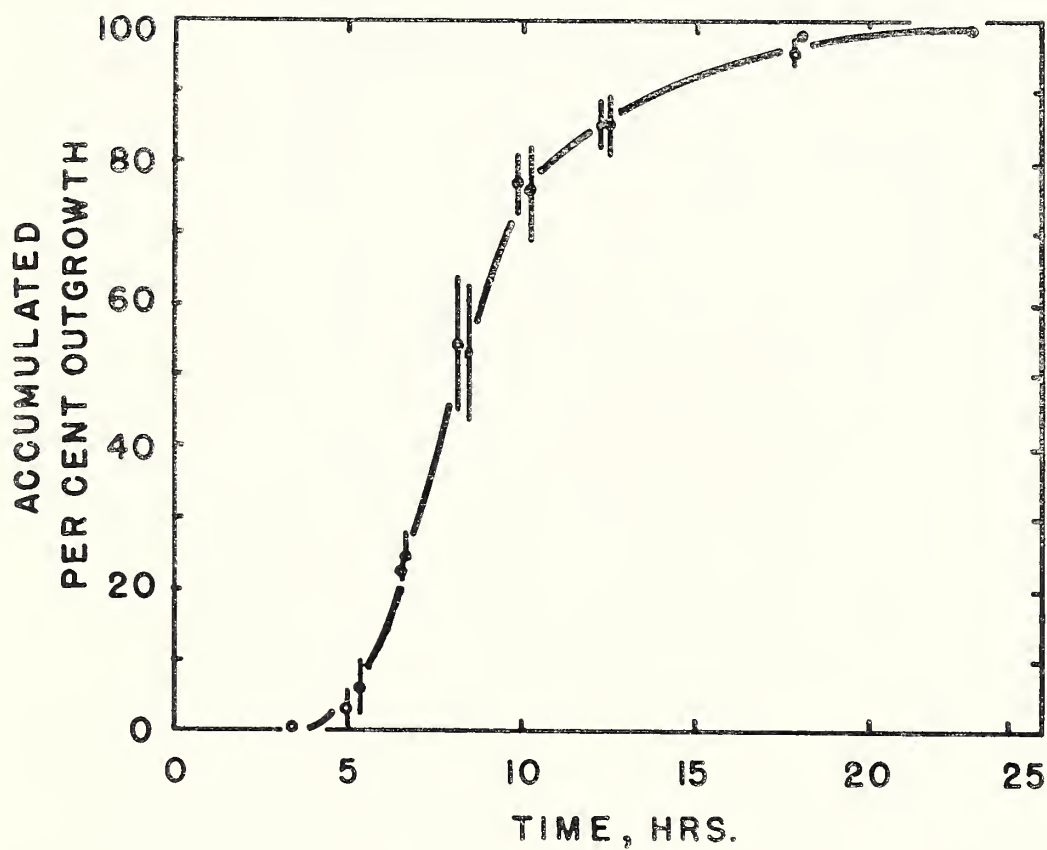


Figure 1. Accumulated percent outgrowth of germ tubes in Cladosporium cucumerinum fungal spores following inhibition and placement on a water agar plate as described in the Materials and Methods. The two symbols are replicate plates, and the vertical lines are one standard deviation. The curve was drawn by inspection.



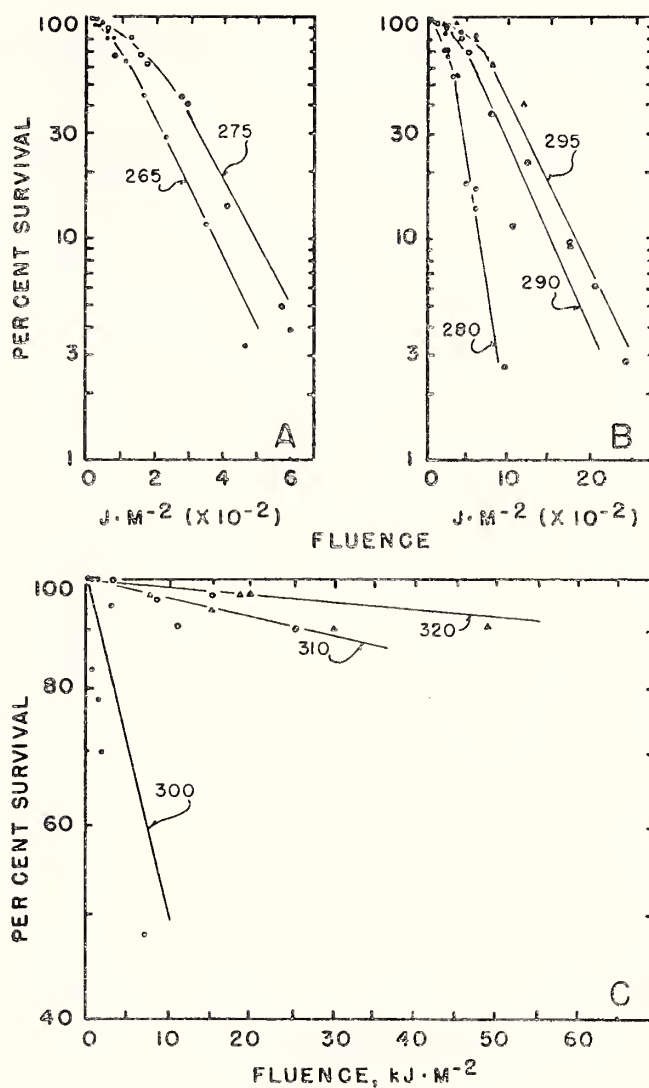


Figure 2. A, B, and C: survival curves for 5 nm half-band width UV exposures of *Cladosporium cucumerinum* fungal spores at the indicated central wavelengths. Percent survival was determined at 22 hours and is the percent of control survival. The fluences were obtained by varying the time.





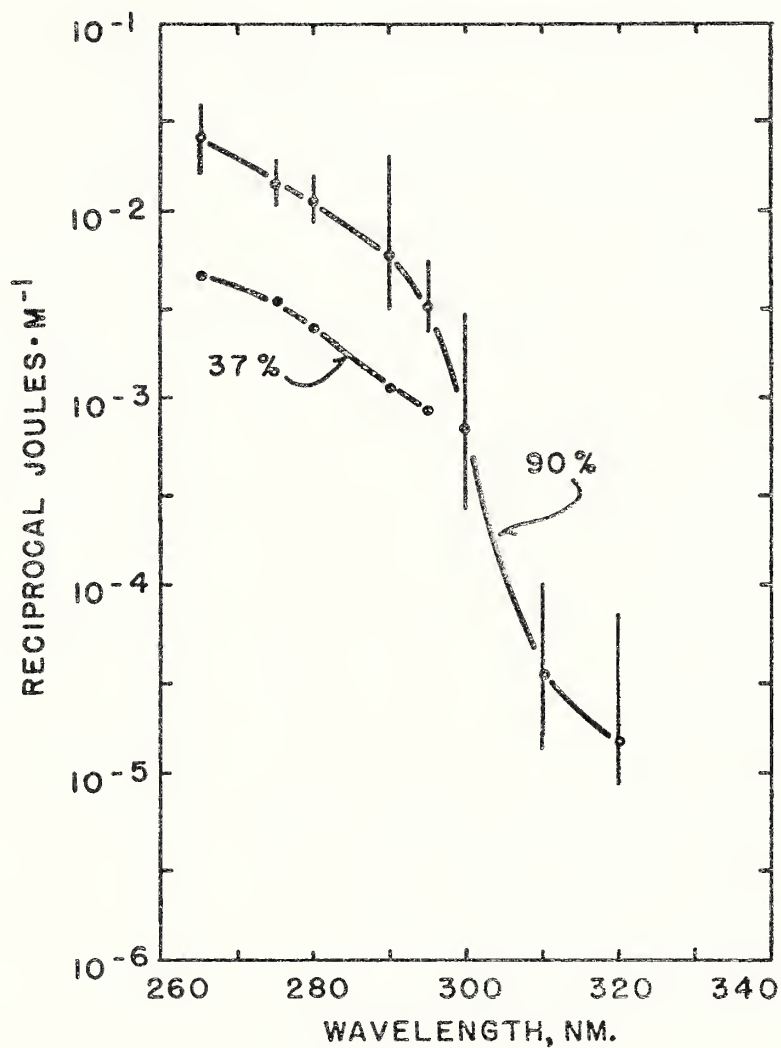


Figure 3. Action spectra for UV inhibition of fungal spore germination in Cladosporium cucumerinum plotted from data of Figure 2 (A, B, and C). Action is expressed as reciprocal of the joules·m<sup>-2</sup> that gave 90% (o) and 37% (•) survival. The vertical lines are the 95% confidence limits.



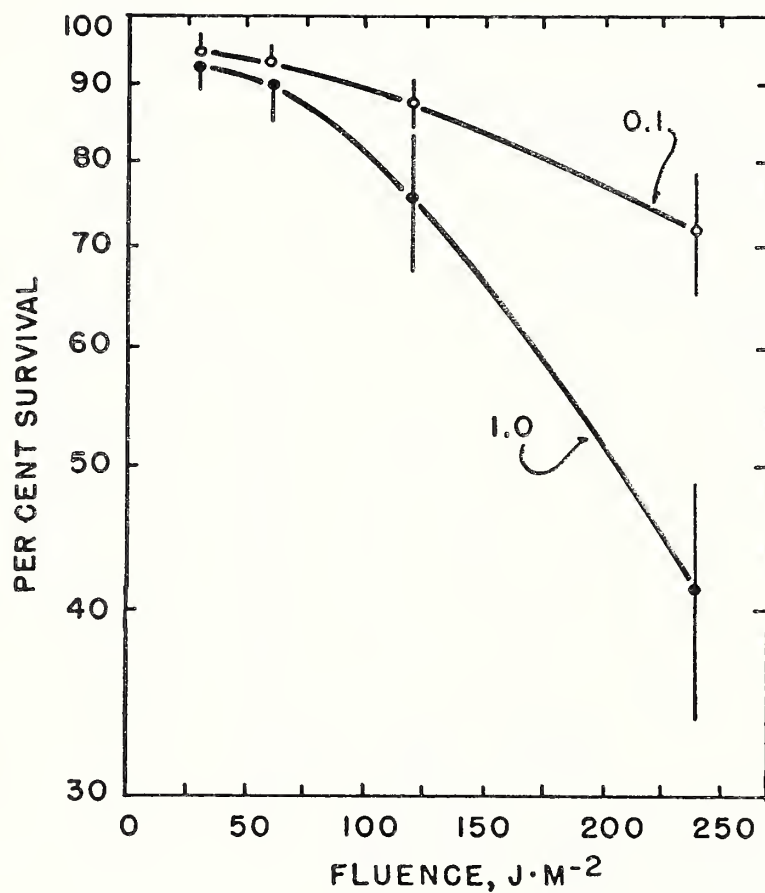


Figure 4. Percent survival of Cladosporium cucumerinum fungal spores following exposure to UV irradiation at 265 nm given at 0.1  $\text{J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  (o) and 1.0  $\text{J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  (●). Counts were made as in Fig. 2.



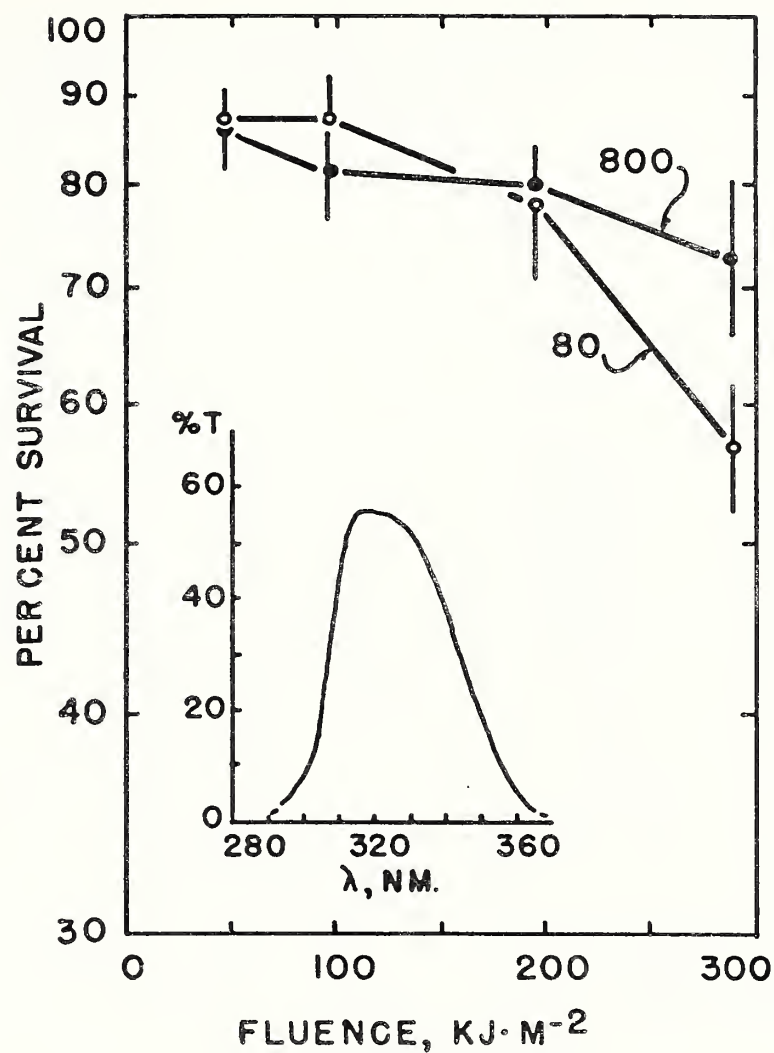


Figure 5. Percent survival of Cladosporium cucumerinum fungal spores following exposure to a broad band UV source centering on 325 nm (inset) given at 80 J·s<sup>-1</sup>·m<sup>-2</sup> (o) and 800 J·s<sup>-1</sup>·m<sup>-2</sup> (●). Counts were made as in Fig. 2.



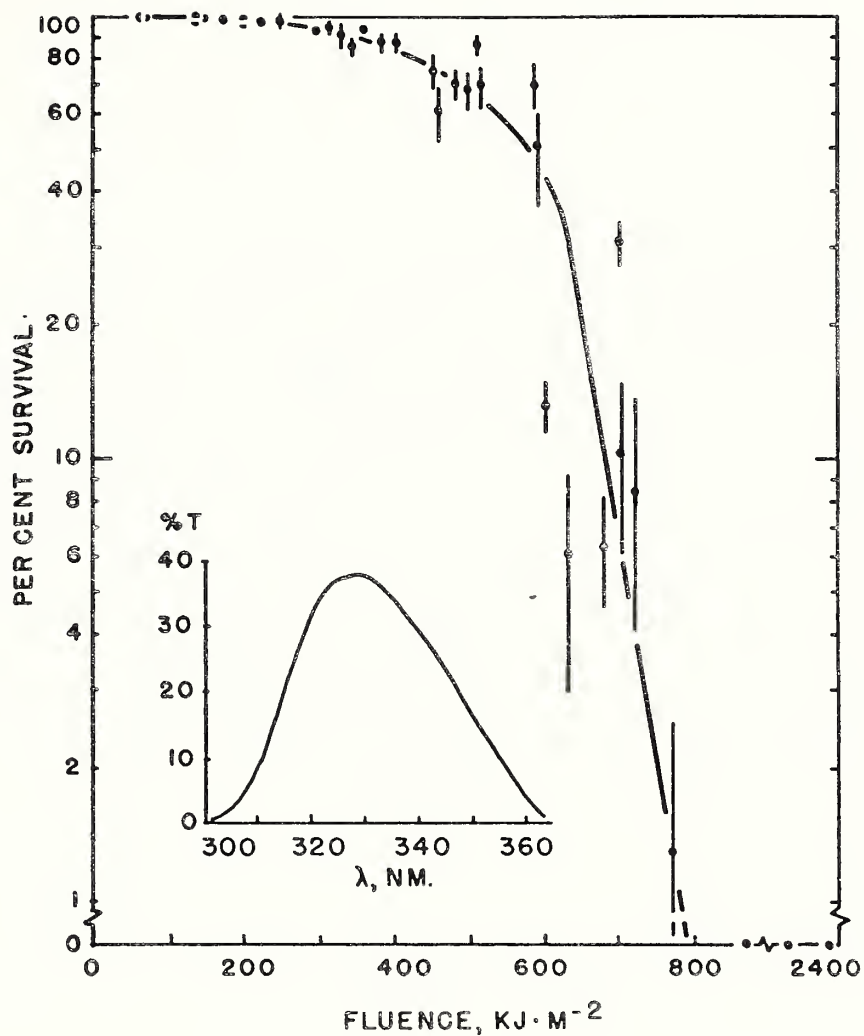


Figure 6. Survival curve for exposure of *Cladosporium cucumerinum* fungal spores to a broad band UV source centering on 330 nm (inset) given at  $400 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ . Counts were made at 22 hours as in Fig. 2.





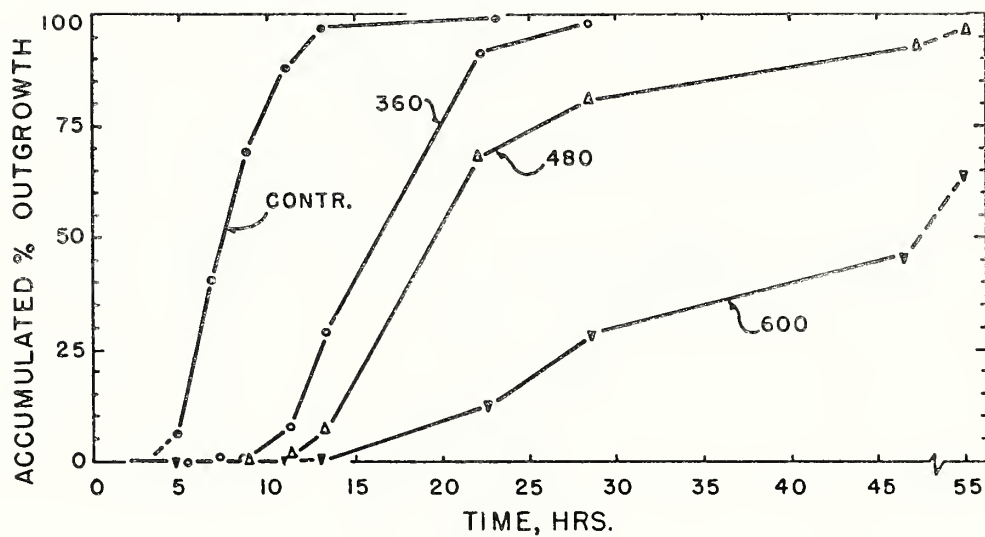


Figure 7. Accumulated percent outgrowth of Cladosporium cucumerinum fungal spores vs duration of exposure to broad band UV irradiation centering at 330 nm. The indicated values (360, 480, and 600), are  $\text{kJ}\cdot\text{m}^{-2}$  obtained by duration of exposure to  $400 \text{ J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  for 15, 20, and 25 min., respectively. Control spores received no UV irradiation.



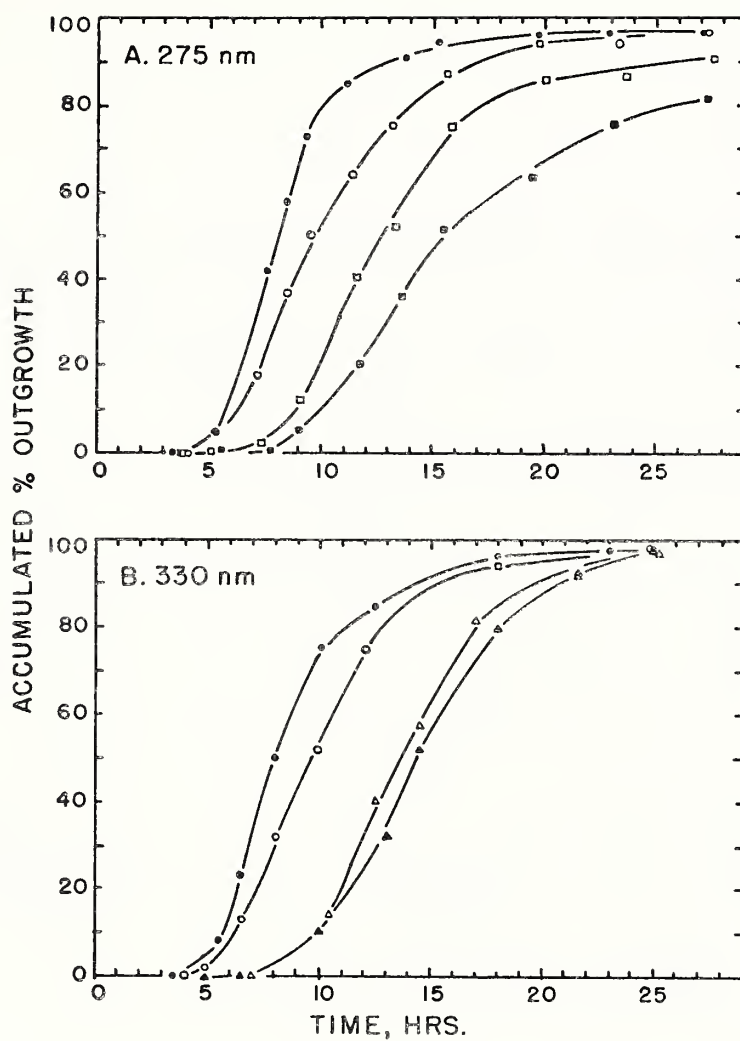


Figure 8. Time course for outgrowth of *Cladosporium cucumerinum* fungal spores showing comparative influence of short-wave and long-wave UV. A. 275 nm narrow band UV (5 min.  $0.57 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ ; ◐, ◑). B. 330 nm broad band UV (5 min.  $5.20 \text{ J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ ; ◐, ◑). Controls (no UV exposure: ●, ○). Plates were incubated in dark (closed symbols) or in light, (open symbols).



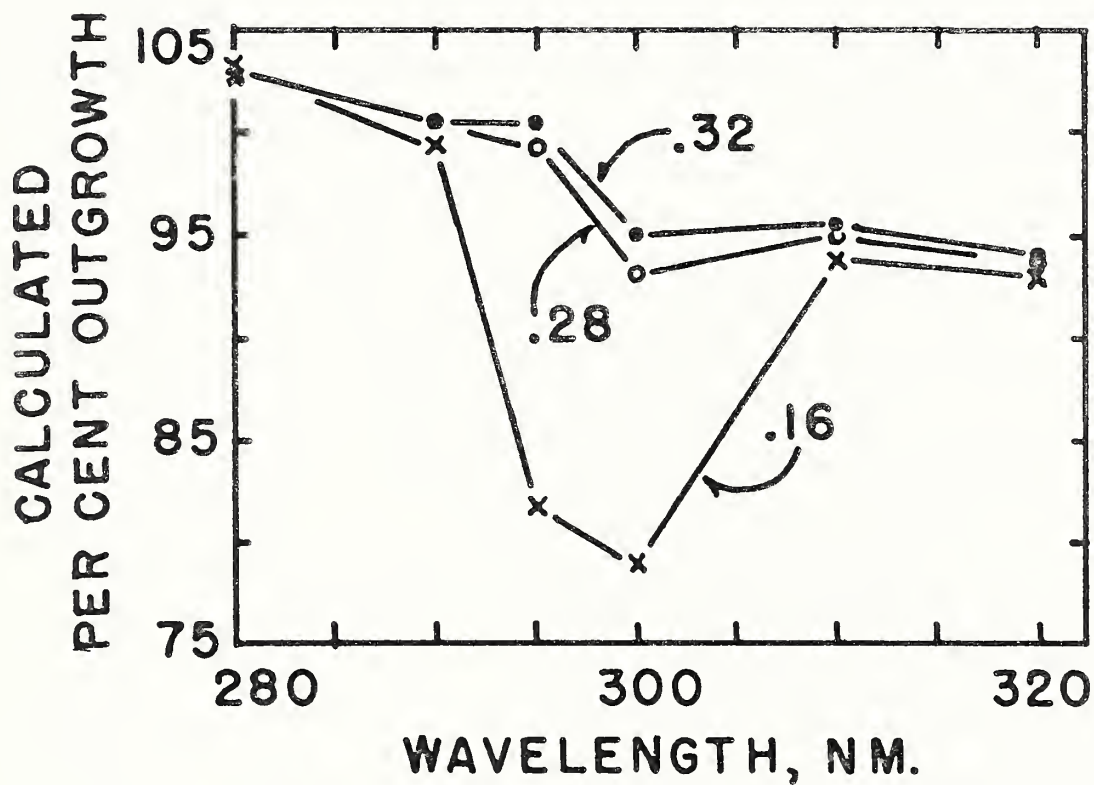


Figure 9. Influence of stratospheric ozone reduction on percent germ tube outgrowth of Cladosporium cucumerinum fungal spores in the UV-B region. Values were calculated from the data of Fig. 2 and Table 2. The values assigned to the curves represent "normal" ozone concentration, 0.32 atm·cm; 12% reduction, 0.28 atm·cm; and 50% reduction 0.16 atm·cm.









FINAL REPORT

RESPONSE OF SELECTED VEGETABLE AND AGRONOMIC CROPS TO  
INCREASED UV-B IRRADIATION UNDER GREENHOUSE CONDITIONS

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## ABSTRACT

Biomass measurements for alfalfa, rice, and wheat in greenhouse experiments showed no reduction in growth of enhanced UV-B treated plants as compared with the Mylar<sup>1/</sup> control. None of these species showed chlorotic tissue. However, the 'Poinsett' cucumber cultivar under 13 to 15 mWm<sup>-2</sup> BUV of UV-B showed chlorosis of about 11 percent of the total leaf tissue, whereas, 'Ashley' showed chlorosis of about 1 percent of tissue on a dry weight basis. Leaf area and weight of both cultivars showed about a 7 percent reduction in response to enhanced UV-B as compared to the Mylar control.

For each species studied, there seemed to be a shade effect due to the experimental design of the set-up. Biomass yields were higher from plants at the perimeters than at the centers of the experimental areas.

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<sup>1/</sup> Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be available.



## INTRODUCTION

Recent greenhouse and growth chamber studies conducted in the Plant Stress Laboratory (PSL) (1, 3, 5, 6, 9) have demonstrated the inhibitory effects of high levels of biologically effective UV (BUV) radiation on plant growth and development of cotton and selected vegetable species.

We conducted our experiments to extend these studies to other plant species including alfalfa, cucumber, rice, and wheat. We examined the effects of UV-B on vegetative growth and biomass production in alfalfa, cucumber, rice, and wheat and on grain production in wheat.

## MATERIALS AND METHODS

The UV-B enhancement facilities were developed in cooperation with the Agricultural Equipment Laboratory (AEL), Beltsville Agricultural Research Center (BARC). Enhancement studies were conducted according to the guidelines established for the BACER program (2, 4). Two lamp fixtures provided UV radiation, each containing two Westinghouse FS-40 fluorescent sunlamps filtered with 5 mil Mylar (UV-A) or 5 mil cellulose acetate (UV-A, -B). The UV lamps were kept 62 cm from the plant canopy, and the lamp fixtures were 60 cm apart. Filters were aged for 6 hours before use and were changed twice weekly (4). We measured UV at every pot location at the start of the experiments and at selected points periodically thereafter with either an Optronics Laboratories, Inc., Model 725, UV-B Radiometer or an Instrumentation Research Laboratory (IRL) UV-B Radiometer (7, 8). Radiometer readings were verified by spectral irradiance determinations (250-369 nm) with an automated spectroradiometer (7, 8) at selected locations in the experimental irradiation areas.

Weighted irradiance levels are reported as  $\text{mWm}^{-2}$  BUV, the biologically effective UV derived from the A29 weighting function, and unweighted



irradiance as  $\text{Wm}^{-2}$  obtained by summing the measured or calculated values at each nanometer from 280-320 nm. Dividing  $\text{mWm}^{-2}$  BUUV by 3.06 (the  $\text{mWm}^{-2}$  BUUV of the Beltsville control sunshine) provides the fraction of BUUV received by each plant relative to that of one control sunshine (10).

Where UV irradiation was obtained by filtering the FS-40 lamps through cellulose acetate (CA), BUUV was limited to the UV-B region (280-320 nm).

For details concerning average control sunshine, spectral characteristics of UV fluorescent lamps and filters, and the weighting function, see the BACER final reports of the AEL and IRL, BARC (8, 10).

Experimental plants receiving UV-B were combined into groups averaging  $7 \text{ mWm}^{-2}$  BUUV ( $3.1 \text{ Wm}^{-2}$ ),  $10 \text{ mWm}^{-2}$  BUUV ( $4.5 \text{ Wm}^{-2}$ ),  $13 \text{ mWm}^{-2}$  BUUV ( $5.8 \text{ Wm}^{-2}$ ) and  $15 \text{ mWm}^{-2}$  BUUV ( $6.7 \text{ Wm}^{-2}$ ) for comparison of the results.

#### Cultural Procedure (General)

Plants were seeded in 12.5-cm white plastic pots containing a commercial peat-vermiculite mix, and they were thinned on emergence as follows: alfalfa, 5 plants; cucumber, 1 plant; rice, 8 plants; and wheat, 8 plants per pot.

Plants were grown in a glass greenhouse with and without supplemental UVB during the summer-fall 1977. They were irradiated daily from emergence for 6 hours from 1000 to 1600 hours.

Plant material was dried in a forced-draft oven at  $70^{\circ}\text{C}$ , weighed and combined into the four groups receiving the average 7, 10, 13, or  $15 \text{ mWm}^{-2}$  BUUV under the CA filter and compared to matched set of control plants placed in like groups for similar numbered positions grown under Mylar filters.

### RESULTS

#### I. UV-B effects on alfalfa (*Medicago sativa* L.)

Procedure: Alfalfa cv. 'Williamsburg' was seeded on August 8, 1977. Biomass was harvested by cutting the plants 5.1 cm above the soil level, 6 weeks, 9 weeks, and 12 weeks from seeding.





Results: The results showed no effect from enhanced UV-B (Table 12).

Table 12. Effects of UV-B on biomass (g) of alfalfa\*

Filter	$7\text{mWm}^{-2}$ UV			$10\text{mWm}^{-2}$ UV			$13\text{mWm}^{-2}$ UV			$15\text{mWm}^{-2}$ UV		
	Harvest - wks.			Harvest - wks.			Harvest - wks.			Harvest - wks.		
	6	9	12	6	9	12	6	9	12	6	9	12
Mylar	5.2	2.7	1.6	3.4	1.8	1.4	4.0	2.2	1.5	3.2	1.7	1.4
CA	6.4	3.0	2.2	4.9	2.1	1.2	4.5	1.9	1.4	3.8	1.9	1.6

\* Mean weight 10 plants.

Discussion: A shade effect was noted--biomass yield was largely influenced by pot position. Plants grown directly under light fixtures produced less growth than plants not directly under the fixture.

## II. UV-B effects on cucumber (*Cucumis sativus* L.)

Procedure: Pots were seeded alternately with 'Ashley' or 'Poinsett' cultivars on November 28, 1977, and thinned to one plant per pot on emergence. Above-ground plant parts, excised at the cotyledonary node were harvested 4 weeks after seeding. Leaf areas were measured and selected plant sections were placed in a 1:1:18 FAA solution for morphological examination.

Results: The effects of increased UV-B on cucumber are shown in Table 13.



Table 13. Effect of UV-B on leaf weight and leaf area in cucumber

$\text{mWm}^{-2}$ UV		Dry weight (g)			
		CA filter		Mylar filter	
		'Ashley'	'Poinsett'	'Ashley'	'Poinsett'
UV-B* irradiation	7	.343	.345	3.67	4.34
	10	.244	.295	3.23	3.84
	13	.235	.244	3.02	3.40
	15	.209	.199	3.06	4.06
		Leaf area			
		CA filter		Mylar filter	
		'Ashley'	'Poinsett'	'Ashley'	'Poinsett'
UV-B* irradiation	7	194	200	203	242
	10	129	161	191	221
	13	132	132	170	193
	15	114	119	176	216

\* Number of samples for 7, 10, 13, and 15  $\text{mWm}^{-2}$  UV were 12, 16, 22, and 6, respectively.

Discussion: Dry weights. Three effects were noted. A shade effect from light fixtures, enhanced UV-B effect and a chlorotic leaf-tissue effect. Yield of control plants (Mylar) directly under light fixtures was about 90 percent of the yield of plants not directly under light fixtures. This was due to a shading effect.

Leaf dry weight of plants grown under the CA filters, as compared with plants at like positions grown under the Mylar filters, were reduced 9, 8, 7, and 5 percent for 'Poinsett' and 9, 8, 8, and 7 percent for 'Ashley', respectively.



Chlorotic leaf tissue in the 'Poinsett' cultivar under  $13-15 \text{ mWm}^{-2}$  UV-B made up about 11 percent of the leaf tissue on a dry weight basis. In 'Ashley' for similar BUV levels chlorotic leaf tissue comprised about 1 percent of the total leaf tissue on a dry weight basis.

Plants grown under Mylar filters (control) appeared green and normal for all pot positions.

#### Leaf Area

Two effects were noted, a shade effect and an enhanced UV-B effect. Leaf area of control plants grown directly under light fixtures was about 90 percent of the values of plants not grown directly under light fixtures. Leaf areas of plants grown under the CA filters, as compared with plants in like positions under Mylar filters, were reduced by 17, 27, 32, and 45 percent for 'Poinsett' and 4, 32, 22, and 35 percent for 'Ashley', respectively, with increasing UV-B demonstrating varietal differences within species.

### III. UV-B effects on rice (*Oryza sativa* L.)

Procedure: Rice was seeded in soil enclosed by a plastic bag within a waxed container on August 8, 1977. Plants were thinned to eight plants per pot and flooded 2 weeks after seeding. Flooded conditions were maintained throughout the experiment. Plants were harvested on December 27, 1977, 19 weeks from seeding.

Results: The results of increased UV-B in rice are shown in Table 14.

Table 14. Effect of UV-B on biomass (g) of rice\*

Filter	<u><math>7\text{mWm}^{-2}</math> BUV</u>	<u><math>10\text{mWm}^{-2}</math> BUV</u>	<u><math>13\text{mWm}^{-2}</math> BUV</u>	<u><math>15\text{mWm}^{-2}</math> BUV</u>
Mylar	21.2	14.3	15.7	10.7
CA	22.3	19.0	15.5	10.8

\* Mean weight per pot.



Discussion: One effect was noted, a shade effect which directly affected the plants growing in the center of the quadrant. Plants on the outer perimeter were not affected by shade and thus were greater in height and growth. The inner plants were shaded by light fixtures and also shaded by plants external to their pot positions.

#### IV. UV-B effects on wheat (*Triticum aestivum* L.)

Procedure: Wheat cv. 'Pacific Triple Dwarf' was seeded in the summer of 1977 and harvested 12 weeks after seedling emergence.

Results: The effects of increased UV-B on vegetative growth and grain development were negligible (Table 15). Plant growth appeared to be green and normal under both Mylar and CA filters.

Table 15. Effect of UV-B on growth and development in Pacific Triple Dwarf wheat from emergence to age 12 weeks.

	<u>Filter</u>	<u><math>7\text{mWm}^{-2}</math> BUUV</u>	<u><math>10\text{mWm}^{-2}</math> BUUV</u>	<u><math>13\text{mWm}^{-2}</math> BUUV</u>	<u><math>15\text{mWm}^{-2}</math> BUUV</u>
Straw					
dry weight (g)	CA	7.72	6.49	7.11	7.27
per 10 plants	Mylar	8.51	7.35	7.04	7.45
Grain, No. seeds	CA	161	137	132	140
per 10 plants	Mylar	209	161	181	161
Grain weight (g)	CA	2.73	2.72	2.56	2.69
per 100 seed	Mylar	2.29	2.37	2.32	2.46





#### LITERATURE CITED

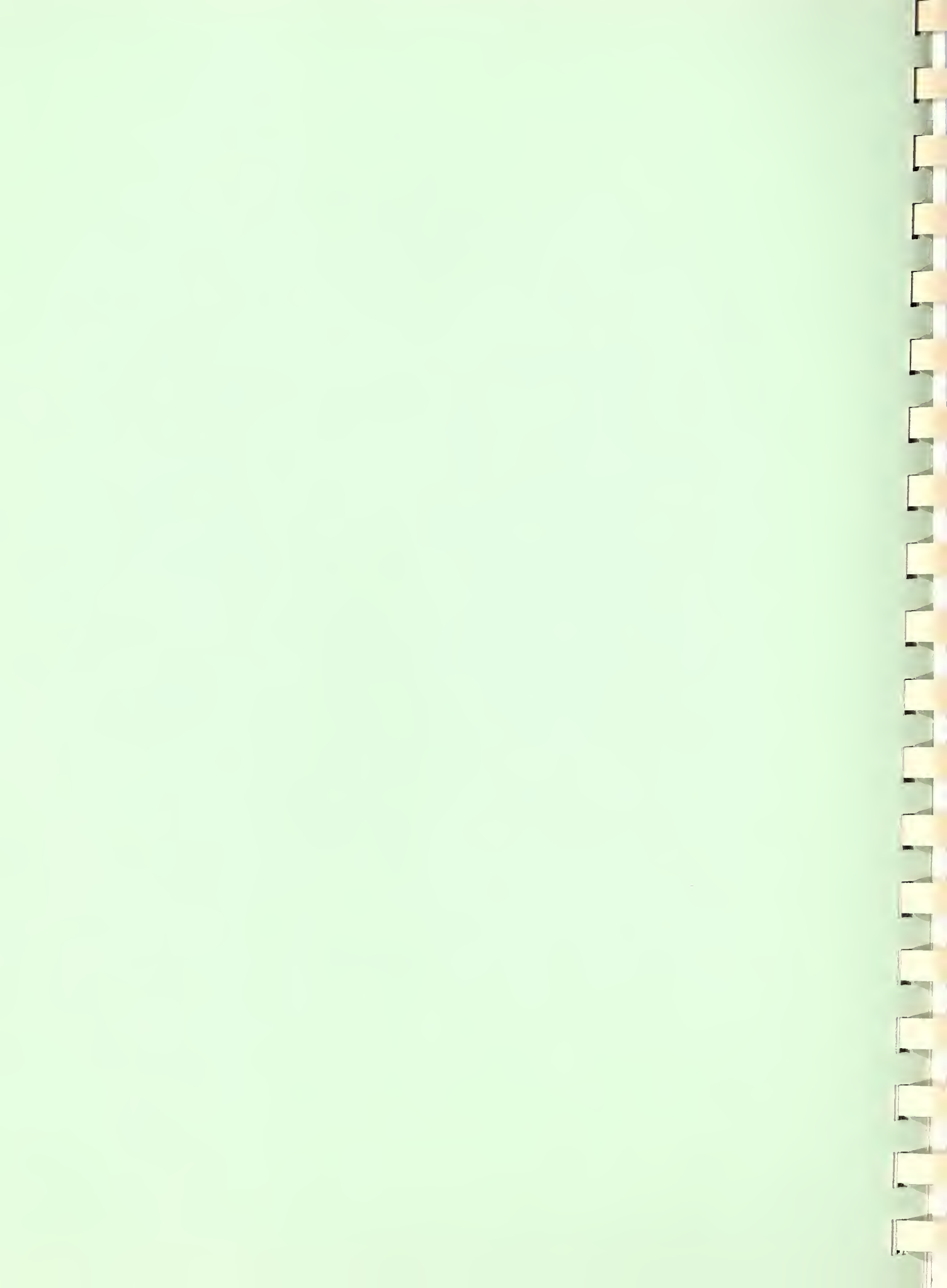
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FINAL REPORT

RESPONSE OF SELECTED VEGETABLE AND AGRONOMIC CROPS TO  
INCREASED UV-B IRRADIATION UNDER FIELD CONDITIONS

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# ABSTRACT

Enhanced UV-B radiation under field conditions on various economic crops conducted at the Beltsville Agricultural Research Center ranged from ambient up to eight times ambient. The enhanced radiation was achieved with either unfiltered BZS-CLG or FS-40 Westinghouse<sup>1/</sup> sunlamps.

All crops were affected less by the enhanced radiation than similar crops grown under greenhouse and growth chamber conditions.

Although variable, these results supported results obtained elsewhere in this BACER Program which indicate that higher plants have a high light energy-requiring system of photorepair or photoprotection. With all crops investigated, additional research is needed to establish the levels of UV-B required to injure plants in the field.

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<sup>1/</sup> Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be available.



## INTRODUCTION

Growth chamber and greenhouse experiments have indicated that several crop plants displayed reduced growth and chlorosis when subjected to enhanced levels of UV-B (1, 2, 3, 4). The field experiments reported here were designed to examine the responses of selected crops to enhanced UV under field conditions.

## MATERIALS AND METHODS

The field selected for these studies was located on the south farm of the Beltsville Agricultural Research Center, Beltsville, Maryland. The plots were silt loam, characteristically consisting of recently deposited materials washed from acid crystallite rock of the capitol Piedmont. These soils also have a concentration of fine mica, which contributes to poor drainage. The area was rototilled in early spring of 1977 and fertilized with a 10-10-10 fertilizer at a rate of 500 pounds/acre 3 weeks before planting.

The total area was divided into six plots, which were supplied with separate UV-enhancement assemblies. The lamp assemblies, constructed by the Agricultural Equipment Laboratory, were designed so that height adjustment could be easily maintained by a simple pulley system (Fig. 1). A height of 1.6 m was maintained above the plant canopy during the course of the experiment.

For the first experiment, lamp banks were designed to obtain a two dimensional gradient of UV irradiation--one parallel to a lamp assembly, the other at right angles to the lamp assembly. Each assembly consisted of lamp fixtures placed end to end as follows: A two-lamp fixture at the high UV-B end; then two single lamp fixtures placed end to end; a 33-cm







Figure 1





space and another single lamp; a 65-cm space and a final lamp that was taped to reduce the UV-B irradiance by one half. This positioning of the lamps provided a uniform gradient of supplemental UV within the experimental plot area. Each plot consisted of four 11.0-m rows parallel to the UV-B gradient. Two rows were 0.5 m from the center line of the lamp assembly, the other two were 1.5 m from the assembly center line. Supplemental UV radiation was provided by unfiltered Westinghouse BZS-CLG 40 watt fluorescent lamps. Exposure time from emergence to harvest was 6 hours/day from 1100 to 1700 hours. Table 1 shows the irradiance levels obtained for each meter of row length.

Weighted irradiance levels are reported as  $\text{mWm}^{-2}$  BUUV, the biologically effective UV derived from the A29 weighting function, and unweighted irradiance as  $\text{mWm}^{-2}$  obtained by summing the measured or calculated values at each nanometer from 280-320 nm. Dividing  $\text{mWm}^{-2}$  BUUV by 3.06 (the  $\text{mWm}^{-2}$  BUUV of the Beltsville control sunshine) provides the fraction of BUUV received by each plant relative to that of one control sunshine (6).

Two different crops were chosen for each plot to minimize shading effects, and they were grown in alternate 0.5- and 1.5-m rows. The paired crops were as follows: squash (Curcubita maxima (L.) cv. 'Early Prolific Straightneck') and bean (Phaseolus vulgaris (L.) 'Contender Bush'); sweet corn (Zea mays (L.) cv. 'Golden Cross Bantum') and sorghum (Sorghum bicolor (L.) Moench cv. 'R-720'); and soybean (Glycine max (L.) Merr. cv. 'Amsoy-71') and sugar beet (Beta vulgaris (L.) cv. '7322-0'). Also included were guard rows of pea (Vigna unguiculata (L.) Walp. cv. 'Cow') on either side of the 1.5-m rows. Each plot of paired crops was duplicated. All plants were sown in May unless otherwise specified.

A second experiment was undertaken in mid-summer using unfiltered Westinghouse FS-40 UV fluorescent lamps. A similar gradient assembly was





Table 1. Spectroradiometer measurements at 1-m intervals along the 11-m rows for BZS-CLG sunlamps unweighted ( $\text{mWm}^{-2}$ ) and weighted ( $\text{mWm}^{-2}$  UV) and fraction of control sunshine above ambient.

Row position (m)	$\text{mWm}^{-2}$	$\text{mWm}^{-2}$ UV	Control sunshine (fraction)
0.5-m Row			
0	9.113	.146	.05
1	96.131	1.669	.55
2	138.105	2.498	.82
3	117.339	2.131	.70
4	94.996	1.736	.57
5	79.977	1.450	.47
6	63.131	1.137	.37
7	42.018	.898	.29
8	28.319	.518	.17
9	7.562	.124	.04
10	1.768	.026	.01
11	.662	.009	.00
1.5-m Row			
0	12.109	.193	.06
1	45.701	.790	.26
2	65.431	1.175	.38
3	76.497	1.553	.51
4	66.966	1.146	.47
5	53.034	.956	.31
6	32.011	.547	.18
7	29.146	.491	.16
8	19.644	.283	.09
9	5.246	.068	.02
10	1.227	.014	.00
11	.459	.004	.00



Table 2. Spectroradiometer measurements at 1-m intervals along the 14-m rows for FS-40 sunlamps unweighted ( $\text{mWm}^{-2}$ ) and weighted ( $\text{mWm}^{-2}$  BUV) and fraction of control sunshine above ambient.

Row position (m)	$\text{mWm}^{-2}$	$\text{mWm}^{-2}$ BUV	Control sunshine (fraction)
0.5-m Row			
0	88.672	3.227	1.05
1	450.287	17.594	5.75
2	709.471	28.736	9.39
3	607.306	24.280	7.93
4	512.496	19.879	6.50
5	421.542	17.035	5.57
6	347.719	14.271	4.66
7	232.890	9.473	3.09
8	163.677	6.763	2.20
9	60.898	2.333	.76
10	17.505	.636	.21
11	8.025	.292	.09
12	4.263	.155	.05
13	1.583	.058	.02
14	.432	.015	.00
1.5-m Row			
0	80.56	3.182	1.03
1	409.287	7.566	2.47
2	310.778	12.357	4.04
3	266.025	11.889	3.89
4	251.797	9.734	3.18
5	207.110	5.132	1.68
6	105.920	4.299	1.40
7	93.942	3.617	1.18
8	80.403	3.296	1.08
9	29.915	1.857	.61
10	13.132	.478	.16
11	6.020	.193	.06
12	3.198	.137	.05
13	1.188	.041	.01
14	.324	.010	.00



constructed with twice as many lamps, which substantially increased the supplemental UV irradiation. Rows were 14 m long. Table 2 shows the irradiance levels obtained for each meter of row length in the plot area.

Radiation was measured with a single monochrometer spectroradiometer described in the Instrumentation Research Laboratory final report (5). Data were obtained at 1-nm intervals from 250 to 369 nm. Experimental results were subjected to analyses of variance.

## RESULTS AND DISCUSSION

Table 3 gives the temperature and precipitation means for the duration of the experiments. There were no unexpected temperature or rainfall extremes, but variability from row to row and within rows was considerable in all experiments, which may account for the lack of statistical significance in many of the parameters measured. Because of this variability, Probability Values (P) of 0.3 and lower are shown for all measured responses.

BZS-CLG lamp assemblies used in the first experiment plus sunshine provided total average irradiance levels as follows; 3.1, 3.7, and 4.2  $\text{mWm}^{-2}$  BUV for the 1.5-m row and 3.2, 4.2, and 5.1  $\text{mWm}^{-2}$  BUV for the 0.5-m row. These irradiances provided approximately 1.0, 1.2, and 1.4 times the Beltsville control sunshine for the 1.5-m row and 1, 1.4, and 1.7 times for the 0.5-m row. The results obtained using these lamps are discussed below on a crop by crop basis:

Squash. Squash was selected as a subject species because its related species, the 'Poinsett' cucumber, was one of the most sensitive crops in Beltsville greenhouse and growth chamber experiments (4). Table 4 shows the effects of UV enhancement on dry weight of tops, fresh weight of fruits, number of fruits, and number of male and female flowers. Only the reductions in fruit weights and number were significant ( $P = 0.2$  to  $0.3$ ). The dry weight of tops also decreased with each increase in UV-B irradiance, but



Table 3. Temperature and Precipitation Measurements, 1977

	Mean high temperature (°C)	Mean low temperature (°C)	Precipitation (mm)
May	25.4	11.8	37.1
June	26.9	14.8	60.2
July	31.7	19.5	112.5*
August	30.7	19.6	42.9
September	27.2	16.3	33.8*
October	18.7	7.3	133.9
November	12.8	5.3	104.9

\* Includes supplemental irrigation





Table 4. Means of squash parameters and standard error for the 0.5-m rows and 1.5-m rows and means and ranges of weighted  $\text{mMm}^{-2}$  for the three levels of biologically effective UV above ambient UV.

Row	Range (mMm <sup>-2</sup> BUV)		Mean (mMm <sup>-2</sup> BUV)	Dry weight of tops (g)	Fresh weight of fruits (g)		Fruits		Flowers		Standard Error		Flowers		Standard Error	
	(mMm <sup>-2</sup> BUV)	(mMm <sup>-2</sup> BUV)			Standard Error	(g)	Standard Error	(No.)	Standard Error	(No.)	Standard Error	(No.)	Standard Error	(No.)	Standard Error	(No.)
Control																
0.5 m	.009-	.518	.165	585.75	567.82	55.4	18.0	1.4	15.62	3.5	4.63	2.1				
1.5 m	.004-	.193	.070	464.89	433.02	110.3	11.0	1.9	6.75	2.5	2.38	0.8				
Low enhancement																
0.5 m	.898-	1.450	1.162	514.63	436.54	126.6	14.3	1.7	14.67	3.3	4.67	1.0				
1.5 m	.491-	.956	.665	357.99	253.95	84.7	7.0	1.1	6.17	2.7	1.67	0.8				
High enhancement																
0.5 m	1.669-	2.498	2.009	485.16	418.59	45.5	13.1	1.7	14.50	2.2	6.38	2.8				
1.5 m	.790-	1.553	1.166	396.53	258.65	25.7	7.9	0.9	6.25	4.7	2.50	0.8				
P =																
0.5 m	N.S.				0.3	0.2		N.S.		N.S.		N.S.				
1.5 m	N.S.				N.S.	0.3		N.S.		N.S.		N.S.				



not significantly. The results suggested that higher irradiance levels may adversely affect plant growth, and continued investigation under field conditions are warranted.

Bean. Garden beans were unaffected by UV-B enhancement. The crop was not visibly damaged during the course of the experiment (Table 5).

Soybeans. Although weight of seed and plant height differed significantly ( $P = 0.2$  and  $0.3$ , respectively) no consistent trends were observed (Table 6). As with beans, the plants were not visibly injured but the results warrant continued investigations.

Sugar beets. This was the most sensitive species tested in the field experiments. All parameters measured except sucrose content at the lower irradiance levels were significantly affected (Table 7). Each UV-B increment reduced dry weight of tops and fresh weight of roots. In contrast, sugar content increased with increased UV-B at the higher irradiance levels, probably as a result of lower metabolic activity in leaves which provided higher carbohydrate concentrations for transport to the roots.

These data, however, suggest that even in field experiments small increases of present ambient UV-B may injure this crop.

Sorghum. The higher irradiance levels reduced fresh weight of tops ( $P = 0.3$ ) (Table 8). Because the probability level was so low and because plant height was not significantly affected, further research is needed to confirm possible UV-B effects.

Sweet corn. The fresh weight of ears, the number of ears, and the plant height were reduced ( $P = 0.2$  to  $0.3$ ) (Table 9). As with the crops other than sugar beets, the level of probability was low. However, since three out of the four parameters measured were significant, continued evaluation of UV-B effects under field conditions seems warranted.



Table 5. Mean of bean parameters and standard error for the 0.5-m rows and 1.5-m rows and means and ranges of weighted  $\text{mWm}^{-2}$  for the three different levels of biologically effective UV above ambient UV.

Row	Range ( $\text{mWm}^{-2}$ BUUV)	Means ( $\text{mWm}^{-2}$ BUUV)	Dry weight of tops (g)	Standard Error	Fresh weight of Fruits (g)	Standard Error	Fruits (No.)	Standard Error
Control								
0.5 m	.009- .518	.165	94.33	8.11	719.0	6.53	15.9	1.33
1.5 m	.004- .193	.070	98.55	4.41	759.4	6.69	15.5	2.44
Low enhancement								
0.5 m	.898-1.450	1.162	97.29	15.70	730.1	4.28	17.0	.61
1.5 m	.491- .956	.665	104.91	2.30	818.7	4.00	17.1	.40
High enhancement								
0.5 m	1.669-2.498	2.009	84.00	6.04	634.2	3.12	12.8	2.11
1.5 m	.790-1.533	1.166	102.25	3.00	789.0	2.92	17.9	.74

Differences were not significant at the 0.3 level according to the P test.



Table 6. Means of soybean parameters and standard error for the 0.5-m rows and the 1.5-m rows and means and ranges of weighted  $\text{mWm}^{-2}$  for the three different levels of biologically effective UV above ambient.

Row	Range ( $\text{mWm}^{-2}$ BUUV)	Mean ( $\text{mWm}^{-2}$ BUUV)	Weight of seed (g)	Standard Error	Height (cm)	Standard Error
Control						
0.5 m	.009- .518	.165	275.88	9.40	476.98	22.2
1.5 m	.004- .193	.070	366.88	36.52	620.65	53.6
Low enhancement						
0.5 m	.898-1.450	1.162	267.73	13.2	470.73	41.5
1.5 m	.491- .956	.665	289.30	13.3	648.57	77.2
High enhancement						
0.5 m	1.669-2.498	2.009	277.15	15.5	541.43	6.1
1.5 m	.790-1.533	1.166	303.63	12.7	518.08	41.1
0.5 m		P =	N.S.		0.3	
1.5 m		P =	0.2		N.S.	





Table 7. Means of sugar beet parameters and standard error for the 0.5-m rows and the 1.5-m rows and means and ranges of weighted  $\text{mWm}^{-2}$  for the three different levels of biologically effective UV above ambient.

Row	Range ( $\text{mWm}^{-2}$ BUV)	Means ( $\text{mWm}^{-2}$ BUV)	Dry weight of tops (g)	Standard Error	Fresh weight of roots (g)	Standard Error	Root sucrose (%)	Standard Error
<b>Control</b>								
0.5 m	.009-.518	.165	470.13	54.3	5705.78	874.7	13.98	0.5
1.5 m	.004-.193	.070	540.33	67.8	5600.00	689.8	14.73	0.2
<b>Low enhancement</b>								
0.5 m	.898-1.450	1.162	295.26	26.7	3720.27	482.9	16.90	0.5
1.5 m	.491-.956	.665	428.21	48.3	3844.97	341.5	14.43	0.0
<b>High enhancement</b>								
0.5 m	1.669-2.498	2.009	243.14	22.2	3059.40	391.2	16.35	0.5
1.5 m	.790-1.533	1.166	388.37	39.8	3997.55	518.1	15.05	0.4
0.5 m		P =	0.05		0.1		0.05	
1.5 m		P =	0.3		0.2		N.S.	



Table 8. Means of sorghum parameters and standard error for the 0.5-m rows and the 1.5-m rows and means and ranges of weighted  $\text{mWm}^{-2}$  for the three different levels of biologically effective UV above ambient.

Rows	Range ( $\text{mWm}^{-2}$ BUUV)	Mean ( $\text{mWm}^{-2}$ BUUV)	Fresh weight of tops (g)	Standard Error	Height (cm)	Standard Error
Control						
0.5 m	.009- .518	.165	2526.68	245.9	1130.15	47.1
1.5 m	.004- .193	.070	2243.23	109.9	852.53	55.1
Low enhancement						
0.5 m	.898-1.450	1.162	2162.23	7.3	1110.30	53.5
1.5 m	.491- .956	.665	2136.63	155.5	866.23	76.1
High enhancement						
0.5 m	1.569-2.498	2.009	2144.05	38.9	1191.40	79.1
1.5 m	.790-1.533	1.166	2163.80	100.3	838.40	59.1
0.5 m		P =	0.3		N.S.	
1.5 m		P =	N.S.		N.S.	



Table 9. Means of sweet corn parameters and standard error for the 0.5-m rows and the 1.5-m rows and means and ranges of weighted  $\text{mMm}^{-2}$  for the three different levels of biologically effective UV above ambient.

Row	Range ( $\text{mMm}^{-2}$ BUV)	Means ( $\text{mMm}^{-2}$ BUV)	Dry weight of tops (g)	Standard Error	Fresh weight of ears (g)	Standard Error	Height (cm)	Standard Error	Ears (No.)	Standard Error
Control										
0.5 m	.009-.518	.165	140.06	14.3	109.40	14.9	140.40	28.8	4.75	.7
1.5 m	.004-.193	.070	185.00	35.0	137.55	14.5	144.35	102.1	5.75	.6
Low enhancement										
0.5 m	.898-1.450	1.162	117.19	13.2	83.42	18.4	146.73	13.3	3.33	.8
1.5 m	.491-.956	.665	196.46	17.8	119.91	17.8	177.83	274.4	5.00	.9
High enhancement										
0.5 m	1.669-2.498	2.009	116.63	11.0	60.97	8.0	128.95	170.6	2.75	.4
1.5 m	.79 -1.533	1.166	142.76	21.9	111.16	13.7	147.15	18.9	4.38	.5
0.5 m		P =	N.S.		0.2		N.S.		0.2	
1.5 m		P =	N.S.		N.S.		0.3		0.3	



For the second experiment, the FS-40 lamp assemblies provided significantly higher total irradiances than those on the first experiment, as well as different spectral characteristics. Irradiances levels averaged 3.8, 5.0, and 11.9  $\text{mWm}^{-2}$  BUV for the 1.5-m row and 3.9, 7.9, and 23.9  $\text{mWm}^{-2}$  BUV for the 0.5-m row. These irradiances provided approximately 1.2, 1.6, and 3.9 times for the 1.5-m row and 1.3, 2.6, and 7.8 times for the 0.5-m row of the control Beltsville sunshine.

Squash. This species was quite resistant to these high irradiance levels. Dry weight of tops and fresh weight of fruit decreased significantly only at the highest irradiances, and then only at  $P = 0.2$  or  $0.3$  (Table 10).

Broccoli. Broccoli was much more sensitive to UV than squash, providing  $P$  values of 0.05 and 0.01 at the higher irradiance levels (Table 11). However, irradiance levels two to eight times the value of the Beltsville control sunshine were required to provide this level of significance.

We conclude from these field experiments that plants are considerably more resistant to injury from enhanced UV in a field environment, with its higher visible light energy, than they are when grown in a greenhouse or a growth chamber. Whether resistance increases enough to preclude UV damage at projected UV-B increases, however, cannot be determined from our results.





Table 10. Means of squash parameters and standard error for the 0.5-m rows and 1.5-m rows, means and ranges of weighted  $\text{mWm}^{-2}$  for the three different levels of biologically effective UV above ambient provided by unfiltered FS-40 lamps.

Row	Range ( $\text{mWm}^{-2}$ BUUV)	Means ( $\text{mWm}^{-2}$ BUUV)	Dry weight of tops (g)	Standard Error	Fresh weight of fruits (g)	Standard Error	Fruits (No.)	Standard Error
Control								
0.5 m	.106- 3.227	.805	444.85	53.0	460.95	62.0	15.6	1.8
1.5 m	.088- 3.182	.743	381.10	54.7	396.83	64.0	15.5	2.8
Low enhancement								
0.5 m	.636- 9.473	4.801	358.95	45.5	342.41	22.3	14.6	2.6
1.5 m	.478- 3.296	1.887	384.09	38.9	302.58	69.2	11.5	1.6
High enhancement								
0.5 m	14.271-28.736	20.840	337.27	30.3	304.69	35.9	14.2	1.3
1.5 m	4.229-12.257	8.797	362.64	55.4	314.89	38.6	14.1	1.6
0.5 m		P =	0.3		0.2		N.S.	
1.5 m		P =	N.S.		N.S.		N.S.	



Table 11. Means of broccoli parameters and standard error for the 0.5-m row and the 1.5-m rows, means and ranges of weighted  $\text{mWm}^{-2}$  for the three different levels of biologically effective UV above ambient provided by unfiltered FS-40 lamps.

Row	Range ( $\text{mWm}^{-2}$ BUUV)	Means ( $\text{mWm}^{-2}$ BUUV)	Dry weight of tops (g)	Standard Error	Fresh weight of Fruits (g)	Standard Error	Fruits (No.)	Standard Error
Control								
0.5 m	.106- 3.227	.805	844.48	79.4	794.82	49.1	3.20	.3
1.5 m	.098- 3.182	.784	977.25	111.9	729.16	155.4	2.50	.3
Low enhancement								
0.5 m	.636- 9.473	4.801	617.33	45.3	605.90	43.9	3.75	.2
1.5 m	.478- 3.296	1.887	849.15	61.2	505.28	59.6	2.50	.4
High enhancement								
0.5 m	14.271-28.736	20.840	602.70	31.7	438.96	53.4	2.30	.3
1.5 m	4.299-12.257	8.797	781.16	21.7	642.34	51.2	2.20	.3
0.5 m		P =	0.05		0.01		0.05	
1.5 m		P =	0.3		N.S.		N.S.	



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FINAL REPORT

EFFECTS OF UV-B RADIATION ON PLANT MEMBRANE PERMEABILITY,  
RESPIRATION AND OXYGEN EVOLUTION

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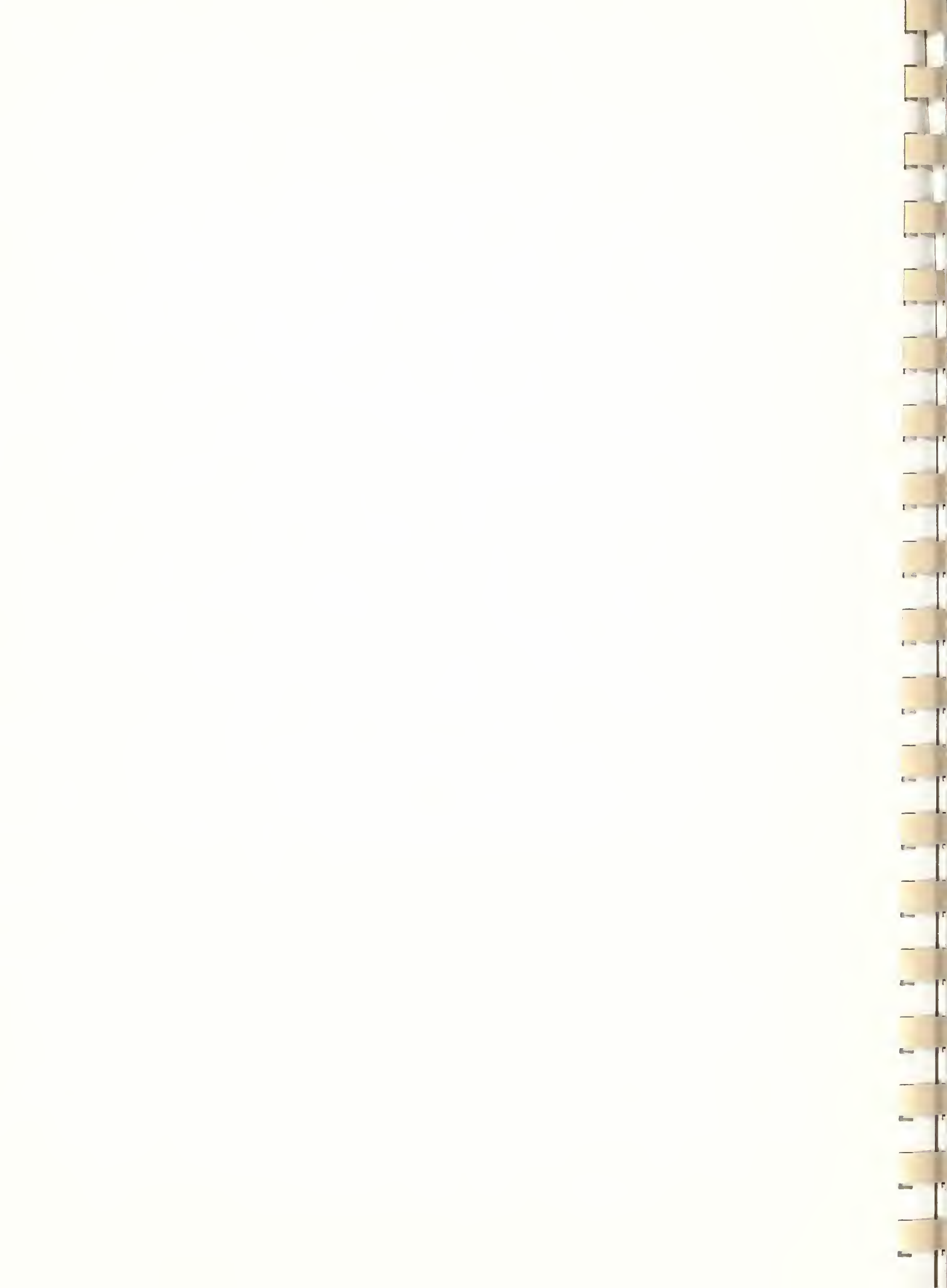
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# ABSTRACT

Poinsett cucumber plants were grown in a growth chamber under UV-A and under UV-A +  $8-24 \text{ mWm}^{-2}$  UV-B (2.8-7.9 Sun equiv.) for 6 hours a day midway in the light period. Leaves of plants under UV-A + UV-B were smaller, weighed less, and took up ions, respired and evolved  $\text{O}_2$  at slower rates than leaves of plants under UV-A alone. The pattern of appearance of these effects varied among leaves and with duration of treatment. The effects of UV-B were much the same whether treatment was started as soon as the plants emerged or on the eighth day of plant growth. When rates of  $\text{Na}^+$  and  $\text{Cl}^-$  uptake, respiration and  $\text{O}_2$  evolution are expressed on the basis of amounts per leaf instead of amounts per gram, the inhibitory effects of UV-B are greater because of the lower weights of the leaves under UV-B. There is no evidence of an increase in ion permeability in these plants under conditions of this study. The data are more indicative of a decrease in permeability insofar as the ion uptake rates were inhibited and somewhat more  $\text{K}^+$  was retained by the plants under UV-B. Effects of respiration,  $\text{O}_2$  evolution and ion uptake generally preceded appearance of visual symptoms (chlorotic spots). Thus measurements of either ion uptake or respiration could provide an early assay for plant sensitivity to UV-B.



## INTRODUCTION

Recent awareness that the use of chlorofluorocarbons in aerosol spray cans and as refrigerants may decrease the protective layer of ozone in the stratosphere sufficiently to cause an increase in ultraviolet radiation in the region of 280-320 nm (UV-B) raises the question of the effect of increased UV-B radiation on agricultural crops. Although effects of ultraviolet radiation in the regions of 100-200 nm (UV-C) and 315-400 nm (UV-A) on plants are well known, there is a dearth of information on effects of UV-B on plants. The work presented here was undertaken to provide information on early effects of UV-B radiation on plants and to assess whether measurements of respiration, oxygen evolution and permeability to ions can serve as assays for UV-B effects before symptoms of toxicity are visible.

## MATERIALS AND METHODS

Seeds of cucumber (Cucumis sativus var. Poinsett) (a cultivar known to be sensitive to UV-B radiation) were germinated on moist paper towels at 30°C. On the second day, each seedling was planted in a 10-cm pot of moistened vermiculite and placed in a growth chamber in the presence or absence of UV-B radiation (Fig. 1). UV-B radiation was from four Westinghouse<sup>1/</sup> FS-40 sunlamps suspended 30 cm above the tops of the plants and covered either with 5-mil Mylar film (UV-A control) or with cellulose acetate at a thickness of 5 mil

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<sup>1/</sup>Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.



(CA-5) or 10 mil (CA-10). The intensity of the UV-B irradiance as measured by a Norris Spectroradiometer is shown in Table 1. The plants were maintained on a 16-hour light period at 30°C with 6 hours of UV-B exposure midway during the light period, and an 8-hour dark period at 25°C. They were watered daily with a 1/5 Johnson solution and the pots were rearranged daily to minimize effects of position. Samples of whole leaves, leaf sections, or cotyledons were taken immediately after UV-B treatment.

Oxygen uptake was measured in the dark and oxygen evolution was measured in the light, in water at 25°C by means of oxygen electrodes. Permeability was assayed by measuring fluxes of  $K^+$ ,  $Na^+$ ,  $Cl^-$ ,  $Ca^{2+}$ , and  $Mg^{2+}$ . Samples were maintained in aerated solutions of  $10^{-2}$  M NaCl +  $10^{-4}$  M  $CaSO_4$  at pH 5.5 for various periods from 1 to 6 hours. Then they were removed, rinsed twice with demineralized water, blotted gently and weighed. The samples were then ashed at 480°C for 1 hour. The ashed samples were dissolved in 1 N  $HNO_3$  + 10%  $CH_3COOH$  and aliquots of these solutions were taken for analyses of ion content.  $Na^+$  and  $K^+$  were determined by flame photometry,  $Ca^{2+}$  and  $Mg^{2+}$  were determined by atomic absorption and  $Cl^-$  was measured by potentiometric titration.

## RESULTS

### Week-Old Cucumber Plants

First leaves of plants grown for 7 days before UV-B treatment was initiated showed little or no difference from controls (under Mylar) in leaf size or weight during the subsequent 2 weeks under UV-B (Table 2). On about the sixth day, small chlorotic areas appeared on the edges of the second and third leaves of the plants under CA-5 filters. Leaves of plants under CA-10 filters had few or no chlorotic spots. Examination of these areas under a microscope revealed





that they were not only devoid of chlorophyll but almost devoid of chloroplasts, starch and other structures as well. No necrotic areas or oxidized phenols were apparent in these leaves when they were examined after removal of chlorophyll.

The second and third leaves of control plants emerged about the third day of UV-B treatment, but emergence and expansion of these leaves under UV-B was delayed initially as shown by relatively low weights at 6 days in Table 2. The weights caught up somewhat when the control leaves reached full development however. Cotyledons tended to decrease in weight sooner under UV-B. The UV-B irradiance had no significant effect for the first 10 days on  $K^+$  concentrations of the first and second leaves whether they were in water or in  $10^{-2}$  M NaCl. However,  $K^+$  concentrations were significantly higher in these leaves at 14 days and at 6 days in the third leaves.

Rates of  $Na^+$  uptake by first leaves under UV-B generally were somewhat stimulated initially (Table 3) but were not effected after 2 days. Although  $Na^+$  uptake by second leaves was inhibited at 6 days, soon after emergence, UV-B had no effect thereafter and had no effect on  $Na^+$  uptake by third leaves. Both  $Na^+$  and  $Cl^-$  uptake by cotyledons under CA-5 was inhibited from the first day of treatment, but UV-B at the lower intensity had no consistent effect. Rates of  $Cl^-$  uptake were more sensitive to UV-B exposure, especially in leaves under CA-5, the higher UV-B intensity. Inhibition was observed as early as 6 days in second leaves and in all leaves by the fourteenth day. Concentrations of  $Ca^{2+}$  and  $Mg^{2+}$  were affected little by UV-B exposure (Table 4). First leaves had higher  $Ca^{2+}$  and  $Mg^{2+}$  concentrations at 14 days than control leaves and third leaves had lower  $Ca^{2+}$  concentrations at 6 days. Rates of oxygen uptake and evolution were also stimulated during the first days of UV-B treatment and inhibited thereafter (Fig. 2). The inhibitory effects were generally greater under CA-5.



### Two-Day-Old Cucumber Plants

Leaves of plants exposed to UV-B from the time of epicotyl emergence (2 days) generally respired and evolved  $O_2$  at somewhat inhibited rates during the first few days after leaf emergence, but thereafter, the rates were about the same as or slightly higher than those of control leaves on a nmole/min-g fresh wt. basis (Fig. 3). Leaves of these UV-B-treated plants, however, weighed less than control leaves (Table 5) and were 30-40% smaller, so that respiration and  $O_2$  evolution on a nmole/plant basis were inhibited. Leaves of plants under UV-B for 8-10 days also took up  $Na^+$  and  $Cl^-$  from  $10^{-2}$  M NaCl at slower rates than control leaves (Table 6). Rates of uptake by first and second leaves were less inhibited by the fifteenth day, suggesting some degree of photorepair. Ion uptake rates by cotyledons were inhibited from the first and showed no evidence of photorepair. Since weights of leaves in these experiments were less also under UV-B, particularly at the higher UV-B intensity (CA-5),  $Na^+$  and  $Cl^-$  uptake rates were likewise inhibited on a micro-equiv/plant basis. There was no consistent effect of UV-B on  $K^+$  concentrations (Table 5). The concentrations in the cotyledons and leaves under UV-B were sometimes higher than those in control leaves; consequently, leakiness in the leaves under UV-B treatment was not evident. Concentrations of  $Ca^{2+}$  and  $Mg^{2+}$  in leaves under UV-B were generally lower than concentrations in control leaves. This probably resulted from inhibition of  $Ca^{2+}$  and  $Mg^{2+}$  uptake from the nutrient solution used to water the plants and not from leakiness. The  $Ca^{2+}$  and  $Mg^{2+}$  concentrations of control leaves increased with age, but concentrations of these ions in UV-B-treated leaves increased very little until about the fifteenth day.



## CONCLUSION

In general, leaves of Poinsett cucumber plants exposed to UV-B radiation in these experiments were smaller, weighed less, and took up ions, respired, and evolved oxygen at slower rates than leaves of control plants under UV-A. The magnitude of the effects reflected the intensity of the UV-B radiation, but did not reflect the age of the plants. Effects of UV-B were much the same whether treatment began on the second day or on the eighth day of plant growth. Weights of the leaves growing under UV-B were lower in both groups of plants, so that expression of the rates of ion uptake, respiration and  $O_2$  evolution and ion contents on the basis of equivalents per plant instead of equivalents per gram would greatly intensify the inhibitory effects. Some degree of photo-repair was suggested insofar as the effects were sometimes less as time proceeded.

There is no evidence of an increase in ion permeability in these studies. The data are more indicative of a permeability decrease since ion uptake rates were inhibited and  $K^+$  was not lost. Effects on ion uptake, respiration and  $O_2$  evolution generally preceded the appearance of visual symptoms. The onset of inhibition of  $Na^+$  and  $Cl^-$  uptake occurred about the same time, regardless of the leaf development, which suggests that the UV-B affected the whole plant. Generally ion uptake was affected at about the same time as respiration and  $O_2$  evolution. This and similarities in the patterns of the effects suggests that ion uptake rates reflect respiratory rates. Thus measurements of either respiration or ion uptake could provide an early assay for plant sensitivity to UV-B radiation.



Table 1. UV-B Irradiances in the Growth Chamber.

Filter	Biologically Effective UV (mWm <sup>-2</sup> )	UV-B Sun Equivalents
Mylar	0.265	0.09
CA 10 Mil.	10.954 - 8.604	3.58 - 2.81
CA 5 Mil.	24.131 - 16.733	7.89 - 5.47

Plants were maintained at 30 cm from the UV source. Pots were rearranged 5x/wk. The higher figures are the irradiance when the filters were new and the lower figures are the irradiance a week later, when the filters were replaced.





Table 2. Effects of UV-B radiation on K<sup>+</sup> concentration and weights of

cucumber leaves						
UV-B Treatment Days	K <sup>+</sup> Concentration			Weight		
	Mylar neq/g	CA-10 % Mylar	CA-5	Mylar g	CA-10 % Mylar	CA-5
<u>Cotyledons</u>						
1	50	108	103	0.55	91	108
2	56	107	112	0.60	108	92
6	61	115	116	0.63	72	77
<u>Leaf 1</u>						
1	91	112	108	0.39	79	91
2	91	103	97	0.62	102	93
6	73	97	103	1.39	90	81
10	62	100	108	1.40	93	101
14	57	117**	112**	1.35	120	119
<u>Leaf 2</u>						
6	85	92	93	2.35	72*	63*
10	66	106	115	2.73	91	91
14	63	119**	111**	3.24	102	88
<u>Leaf 3</u>						
6	62	138**	152**	1.18	103	49*
10	81	96	100	3.70	87	64
14	73	107	113	4.46	101	83

Plants were 7 days old when UV-B irradiance was started. The \* and \*\* indicate significant differences from controls (Mylar) at the 5% and 1% levels, respectively.



Table 3. Effect of UV-B radiation on rates of  $\text{Na}^+$  and  $\text{Cl}^-$  uptake by cucumber leaves from solutions of  $10^{-2}\text{M}$  NaCl, pH 5.5.

UV-B Treatment	$\text{Na}^+$ Uptake Rates				$\text{Cl}^-$ Uptake Rates		
	Mylar	CA-10	CA-5		Mylar	CA-10	CA-5
Days	neq/min-g	% Mylar			neq/min-g	% Mylar	
<u>Cotyledons</u>							
1	31	115	78		19	102	68
2	18	55	53		9	100	72
6	35	111	65		22	104	79
<u>Leaf 1</u>							
1	30	103	121		18	77	122
2	42	117	136		27	103	99
6	36	116	108		20	119	122
10	41	127	88		19	107	91
14	44	97	82		24	67	71
<u>Leaf 2</u>							
6	45	78	76		28	86	72
10	61	93	82		21	95	77
14	54	82	88		32	63	61
<u>Leaf 3</u>							
6	51	99	95		18	112	115
10	51	86	101		23	85	63
14	53	95	93		25	73	59

The plants were 7 days old when UV-B irradiance was started.



Table 4. Effect of UV-B radiation on  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentration of cucumber leaves.

UV-B Treatment Days	$\text{Ca}^{2+}$ Concentration			$\text{Mg}^{2+}$ Concentration		
	Mylar neq/g	CA-10 % Mylar	CA-5	Mylar neq/g	CA-10 % Mylar	CA-5
<u>Cotyledons</u>						
1	103	130	113	85	115	100
2	110	85	138	85	86	118
6	190	137	133	133	114	121
<u>Leaf 1</u>						
1	77	90	75	36	102	86
2	83	88	88	45	106	93
6	151	88	95	84	89	96
10	165	120	134	110	114	98
14	140	160**	184**	84	126	144*
<u>Leaf 2</u>						
6	59	81	81	56	82	85
10	170	90	87	105	91	85
14	187	120	105	98	105	104
<u>Leaf 3</u>						
6	47	45**	35**	47	92	89
10	75	110	90	73	97	90
14	141	110	88	83	106	88

Plants were 7 days old when UV-B irradiance was started. The \* and \*\* indicate significant differences from controls (Mylar) at the 5% and 1% levels, respectively.



Table 5. Effects of UV-B radiation on K<sup>+</sup> concentration and weights of  
cucumber leaves

UV-B Treatment	K <sup>+</sup> Concentration			Weight		
	Mylar	CA-10	CA-5	Mylar	CA-10	CA-5
	neq/g	% Mylar		g	% Mylar	
<u>Cotyledons</u>						
1	61	137**	105	0.196	93	98
2	69	102	100	0.236	116	110
3	61	102	88	0.412	61*	62*
4	76	81	81	0.458	98	93
5	62	98	108	0.515	98	83**
8	60	119	117	0.565	97	79**
10	51	121**	149*	0.492	115	95
<u>Leaf 1</u>						
5	88	103	116	0.385	68	53*
8	72	141**	132*	0.710	79**	63**
10	71	117**	123**	0.812	94	80**
12	66	83	79	0.942	89	72**
15	52	99	114	0.975	97	72**
<u>Leaf 2</u>						
8	91	127*	106	0.762	68**	48**
10	70	124	113	1.690	78*	62**
12	88	75	73	2.065	62*	51**
15	51	111	121*	2.170	93	49**
<u>Leaf 3</u>						
10	99	117	123	0.685	83	51**
12	109	75	81	1.137	88	52*
15	57	95	112	2.848	71*	59**

Plants were 2 days old when UV-B irradiance was started. The \* and \*\* indicate significant differences from controls (Mylar) at the 5% and 1% level, respectively.





Table 6. Effect of UV-B radiation on rates of  $\text{Na}^+$  and  $\text{Cl}^-$  uptake from  $10^{-2}$  M NaCl (pH 5.5) by leaves of cucumber seedlings

UV-B Treatment	Na <sup>+</sup> Uptake Rates			Cl <sup>-</sup> Uptake Rates		
	Mylar	CA-10	CA-5	Mylar	CA-10	CA-5
	Days	neq/min-g	% Mylar	neq/min-g	% Mylar	
<u>Cotyledons</u>						
1	49	90	75	9.2	100	100
2	40	66	58	11.7	-	79
3	35	91	67	9.2	86	77
4	33	83	66	9.6	78	72
5	24	100	84	12.5	73	97
8	28	76	82	14.2	82	50
10	18	96	77	12.3	81	74
<u>Leaf 1</u>						
5	36	132	104	16	91	109
8	47	84	77	20	105	103
10	50	82	75	18	113	84
12	24	88	77	17	110	64
15	38	84	-	20	81	94
<u>Leaf 2</u>						
8	50	73	69	17	64	62
10	57	89	87	26	84	78
12	75	74	68	24	88	91
15	103	94	66	36	91	91
<u>Leaf 3</u>						
10	32	108	80	13	98	72
12	118	52	37	20	88	90
15	132	95	72	52	57	50

Plants were 2 days old when UV-B irradiance was started.



Table 7. Effects of UV-B radiation on  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations of cucumber leaves.

UV-B Treatment Days	$\text{Ca}^{2+}$ Concentration			$\text{Mg}^{2+}$ Concentration		
	Mylar neq/g	CA-10 % Mylar	CA-5	Mylar neq/g	CA-10 % Mylar	CA-5
<u>Cotyledons</u>						
1	65	101	93	76	96	93
2	135	91	88	89	93	95
3	106	94	88	92	91	92
4	175	92	80*	104	100	90
5	123	98	94	109	103	112
8	168	107	95	131	103	100
10	228	91	95	164	94	87
<u>Leaf 1</u>						
5	203	103	99	55	84	71**
8	123	87	84	71	107	97
10	172	97	90	97	99	93
12	260	79	61	127	92	49*
15	250	81	83	123	100	77
<u>Leaf 2</u>						
8	68	90	72*	51	73**	77*
10	122	73**	78**	67	80*	78**
12	296	71	51*	87	96	77
15	197	81	92	134	89	86
20	316	106	90	115	107	82
<u>Leaf 3</u>						
10	81	98	106	52	93	85
12	127	84	80	58	90	96
15	169	91	97	88	89	100
20	231	114	90	75	111	83

Plants were 2 days old when UV-B irradiance was started. The \* and \*\* indicate significant differences from controls (Mylar) at the 5% and 1% levels, respectively.



## FIGURE LEGENDS

Fig. 1. Diagram of the growth chamber showing levels of visible light.

Positions of the UV lamps are indicated by dashed lines. Sheets of methylmethacrylate, shown by solid lines, divided the chamber into 3 sections for the UV-B treatments. The treatments are indicated at the top of the diagram by descriptions of the filters which covered the UV lamps. Initially, there were about 20 pots of plants located in the center of each section.

Fig. 2. Effects of UV-B radiation on rates of  $O_2$  uptake and evolution by leaves of cucumber plants. The rates are expressed as percentages of the control rates (nmoles/min-g fresh wt.). Solid lines are for treatment under CA-10 filtered UV-B and dashed lines for CA-5 filtered UV-B. The plants were 7 days old when UV-B irradiance began.

Fig. 3. Effects of UV-B radiation on rates of  $O_2$  uptake (Fig. 3a) and evolution (Fig. 3b) by leaves of cucumber plants. The rates are expressed as percentages of the control rates (nmoles/min-g fresh wt.). The plants were 2 days old when UV-B irradiance began.



# Visible Light — 43in

5 mil CA	10 mil CA	Mylar
300	310	310
330	330	330
305	320	310

Fig. 1





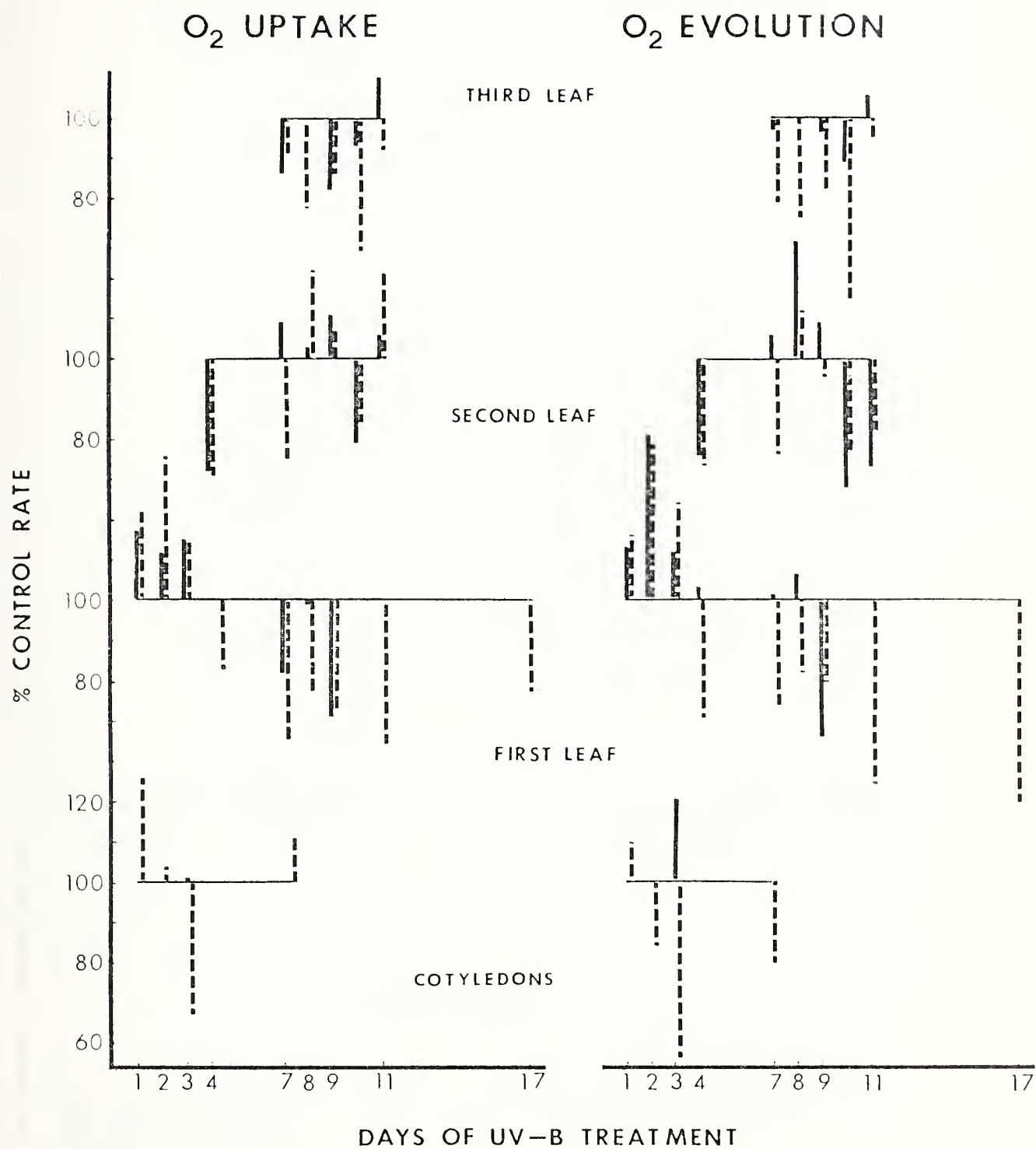


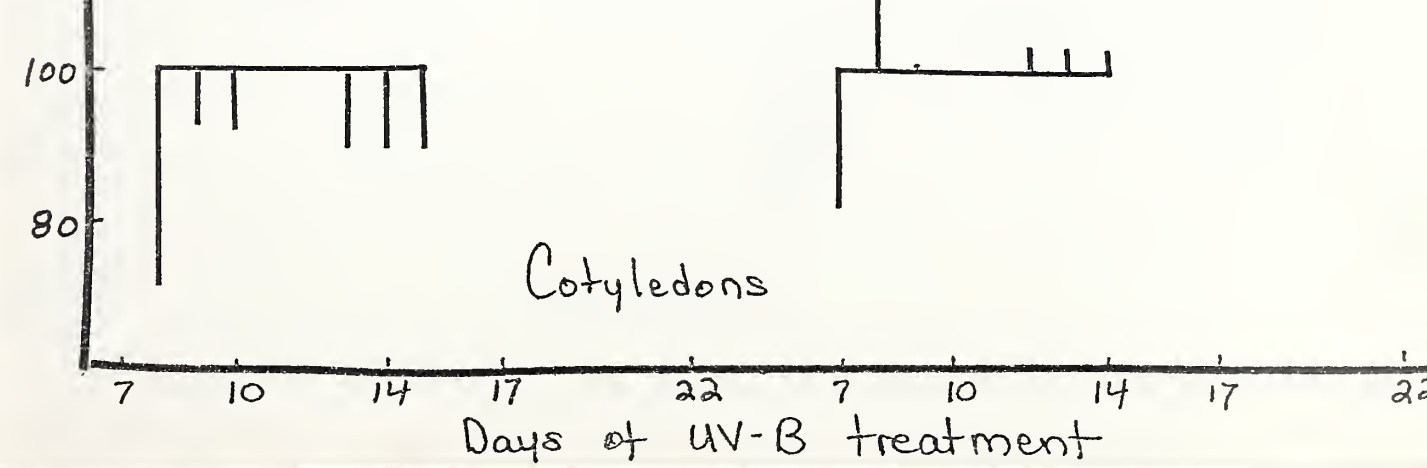
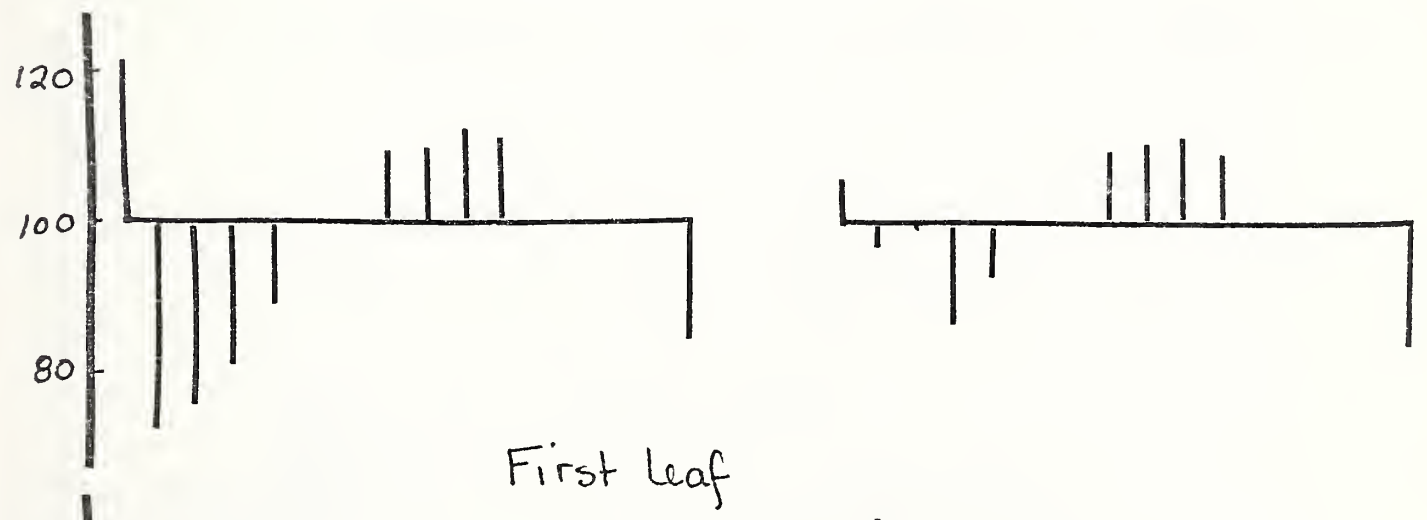
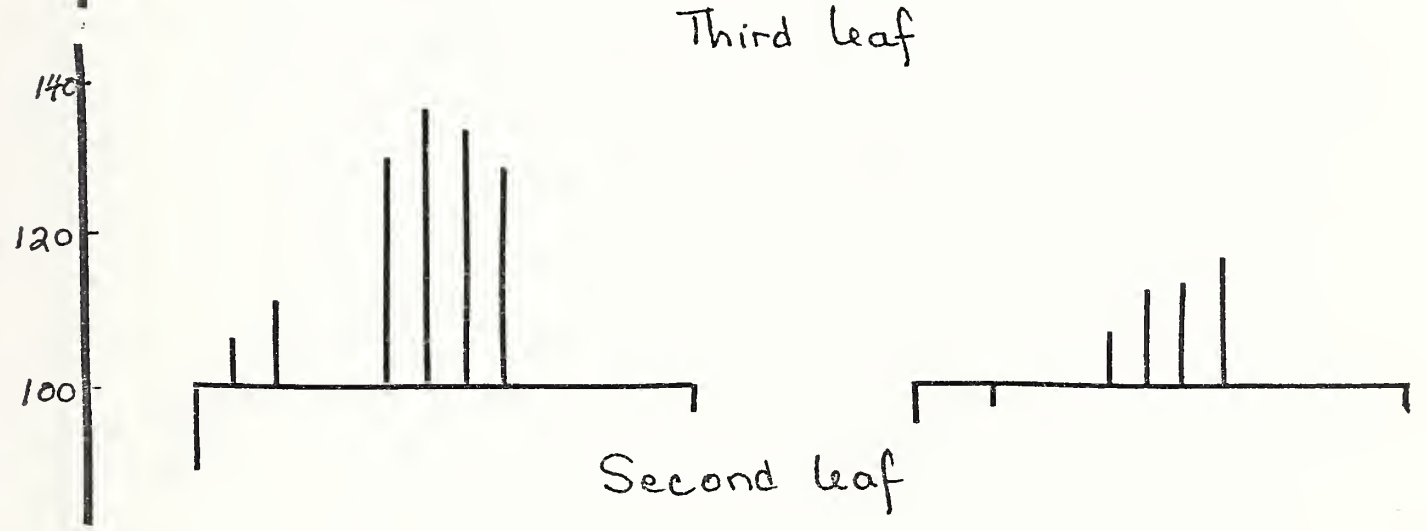
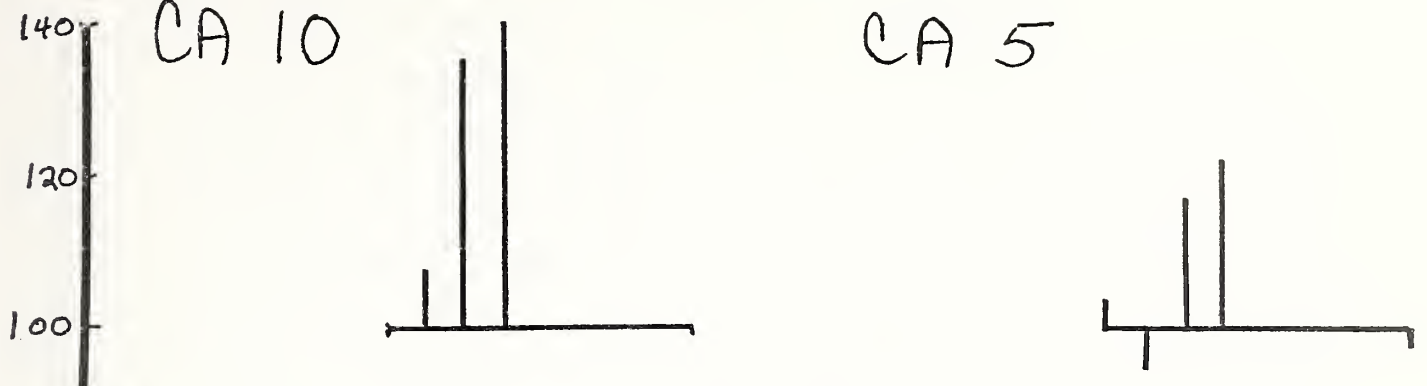
Fig. 2



# O<sub>2</sub> Uptake

CA 10

CA 5



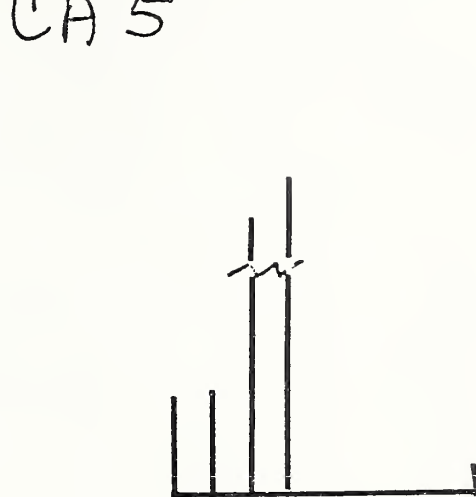
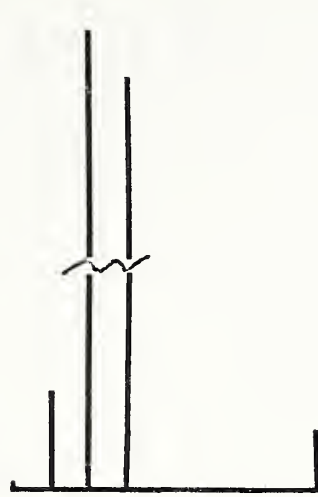
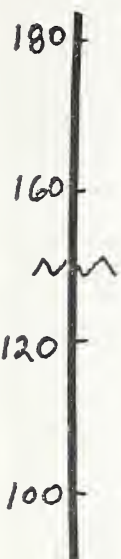
Days of UV-B treatment



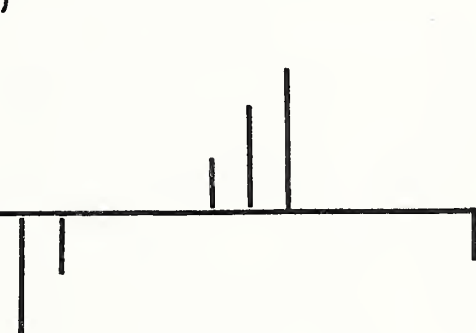
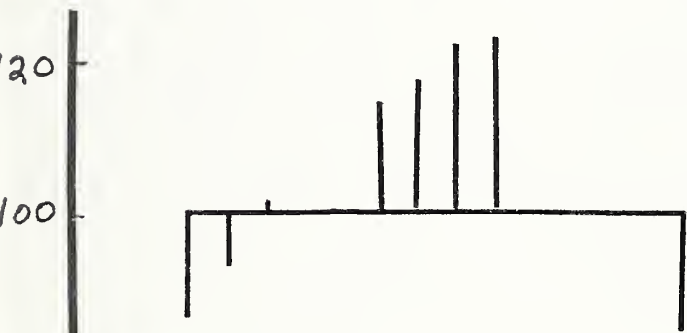
# O<sub>2</sub> Evolution

CA 10

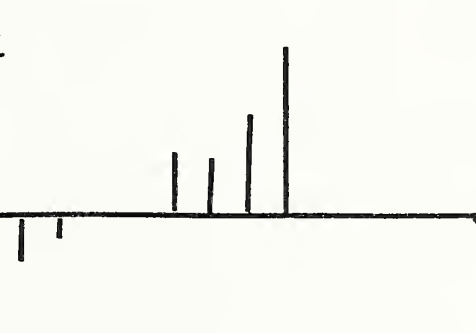
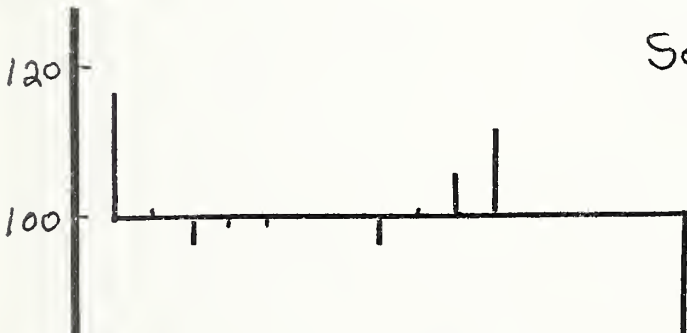
CA 5



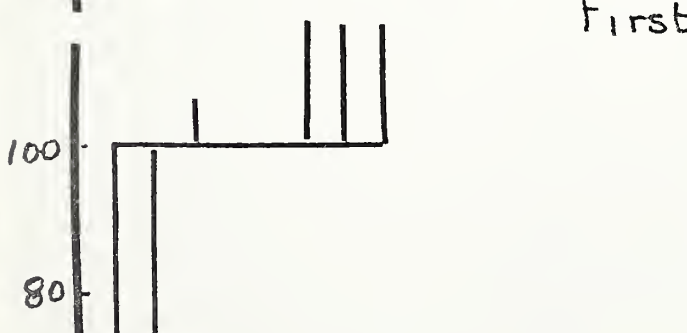
Third leaf



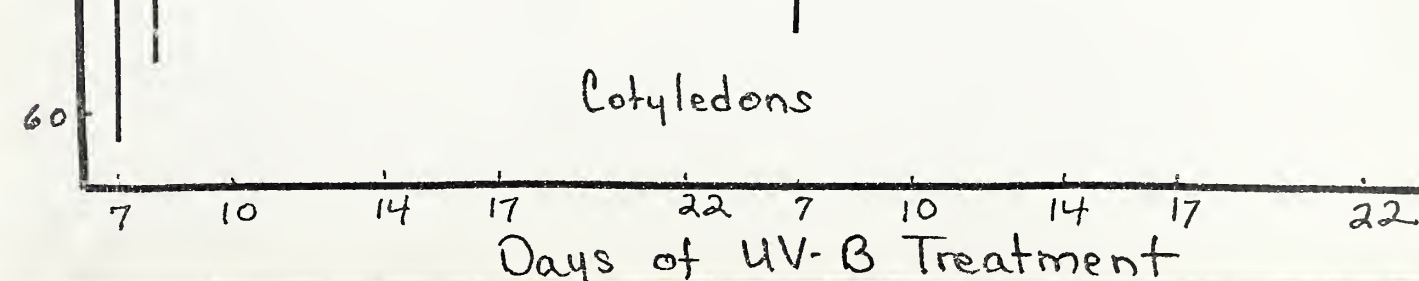
Second leaf



First leaf



Cotyledons



Days of UV-B Treatment









FINAL REPORT

PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS ON UV RADIATION:  
CHANGES IN ANTHOCYANIN PIGMENTATION IN COLEUS BLUMEI BENTH.

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## ABSTRACT

Rooted cuttings of Coleus blumei Benth. were exposed to enhanced UV radiation [50 to 800% increase in biologically effective UV (BUV) radiation] for 1 to 48 hours. The UV radiation was provided by Westinghouse FS-40 fluorescent sunlamps, unfiltered (UV-A, B, C) or filtered with 5, 10 or 20 mil cellulose acetate (UV-A, B) or 5 mil Mylar (UV-A). Exposure to high levels of unfiltered UV radiation (700 to 800% increase in BUV) resulted in a significant decrease in the concentration of anthocyanin extracted from the leaves with methanol and HCl. Degradation of the pigment occurred after 12 hours of exposure and was intensified with an increase in duration of exposure up to 24 hours. Exposure to a 100% increase in BUV under 5 mil cellulose acetate caused glazing of the leaf surfaces, distortion of the leaf margins, and inhibition of leaf expansion. At this UV level, a significant decrease in the anthocyanin content of leaves occurred after 36 hours of exposure (6 days at 6 hours/day).

A leaf injury index was developed to provide a visual evaluation of the extent of UV injury. This index was useful in rating the severity of plant responses to UV treatments at increases in BUV of less than 100%. Spectrophotometric analysis of methanol-extractable materials in coleus leaves indicated that the components absorbing at 280, 330, 412-434, and 525 nm decreased with UV treatment, whereas those absorbing at 415-425 and 650-660 nm increased.



Recent studies (1, 7, 8, 9, 12, 13) have demonstrated that exposure to UV radiation can alter levels of anthocyanin and associated plant pigments. Wellmann (12, 13) showed that UV irradiation induced flavonoid synthesis and phenylalanine ammonia-lyase (PAL) activity in parsley seedlings. Ambler et al. (1) induced red pigmentation in cotton seedlings by exposing them to UV-B radiation. Semeniuk (8) reported that the leaves of 'Supreme Annette' poinsettia formed a purple red anthocyanin when exposed to a 100 percent or greater increase in biologically effective UV (BUV) radiation. In contrast, high levels of UV-B radiation significantly decreased the anthocyanin content of 15 Coleus cultivars (8). Time-lapse photography of Coleus blumei Benth. plants (unpublished data, this laboratory) revealed that the anthocyanin pigment broke down within 24 hours of exposure to broad-band UV radiation from unfiltered Westinghouse FS-40 sunlamps.<sup>1</sup> The extent of the color change was a function of the physiological age of the leaves and the total UV irradiance. In addition, no degradation of the pigment was noted in portions of leaves that were shaded by the plant canopy.

In subsequent experiments, the UV radiation of FS-40 lamps was filtered with cellulose acetate (CA) to approximate the natural UV spectrum (11). The spectral cutoff and total UV irradiance were controlled by varying the filter thickness.

The purpose of this study was to describe the time course of the anthocyanin pigment changes in coleus and to obtain an indication of the metabolic effects of broad band UV radiation.

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<sup>1</sup>Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.



## MATERIALS AND METHODS

UV-B enhancement facilities were developed in cooperation with the Agricultural Equipment Laboratory (AEL), Beltsville Agricultural Research Center (BARC). Enhancement studies were conducted in accordance with the guidelines established for the BACER program (2, 3). UV radiation was provided by one or more Westinghouse FS-40 fluorescent sunlamps unfiltered or filtered with 6-hour-aged 5 mil Mylar (UV-A) or 5, 10, or 20 mil cellulose acetate (UV-A, B).

UV-B irradiance levels were determined with either an Optronic Laboratories, Inc. Model 725 UV-B Radiometer or an Instrumentation Research Laboratory (IRL) UV-B Radiometer (4, 6). Radiometer readings were verified by spectral irradiance determinations (250-369 nm) with an automated spectroradiometer (4, 6) at selected locations in the experimental irradiation areas.

Weighted irradiance levels are reported as  $\text{mWm}^{-2}\text{BUV}$ , the biologically effective UV derived from the  $\lambda\epsilon_9$  weighting function, and unweighted irradiance as  $\text{mWm}^{-2}$  obtained by summing the measured or calculated values at each nanometer from 280-320 nm. Dividing  $\text{mWm}^{-2}\text{BUV}$  by 3.06 (the  $\text{mWm}^{-2}\text{BUV}$  of control sunshine) provides the fraction of BUV received by each plant relative to that of one control sunshine.

When UV irradiation was obtained by filtering the FS-40 lamps through cellulose acetate, BUV was limited to the UV-B region (280-320 nm).

For details concerning average control sunshine, spectral characteristics of UV fluorescent lamps and filters, and the weighting function, see the BACER final reports of the AEL and IRL, BARC (6, 11).

The first experiment (Expt. 1) was conducted with unfiltered UV radiation to determine the change of anthocyanin in the leaves of coleus





with time. The subsequent experiments (Expts. 2 and 3) were conducted with filtered UV radiation to determine the effect of UV radiation on the spectra of methanol-extractable substances in coleus leaves, and to assess the reliability of the anthocyanin content as an indicator of UV damage.

#### EXPERIMENT 1: EFFECT OF UV EXPOSURE ON ANTHOCYANIN PIGMENT OF COLEUS LEAVES

Six-week-old coleus cuttings were exposed to broad-band UV radiation in a laboratory maintained at  $26 \pm 2^{\circ}\text{C}$ . The light sources were one unfiltered FS-40 sunlamp and three 40 watt cool-white fluorescent lamps, positioned 20 cm above the plants to provide approximately  $150\text{--}200 \mu\text{Em}^{-2}\text{s}^{-1}$  of photosynthetically active radiation (PAR). Treatments consisted of continuous exposures of 1, 2, 4, 8, 12, and 24 hours. A UV control was run concurrently under a section of the same lamp bank fitted with a plexiglass filter; this filter has a cutoff at approximately 340 nm. Plants (54 cm in height) were trimmed to two pairs of leaves at the 4th and 5th nodes. The stem was excised above the 5th node. Each pair of leaves was analyzed as a separate sample.

Following UV exposure fresh weights were determined and the leaves were frozen at dry ice temperatures. After 15 minutes, the samples were thawed and cut into strips in preparation for extraction (5). Following extraction with 100 ml of a solution of methanol containing HCl (99:1), the anthocyanin content was measured at 525 nm with a Gilford spectrophotometer. Pigment concentration was reported as absorbance/gm fresh weight.

To determine the effect of shading, several leaves of a separate set of plants were partially shielded by cardboard masks. The change in anthocyanin was observed and recorded photographically.

Because of the large variation in the initial concentration of anthocyanin the experiment was repeated with changes in the sampling and analytical methods.



Instead of using entire leaves as analytical samples, leaf disks (11 mm diameter) were taken from each leaf half. Samples were taken at 0, 12, or 24 hours. These treatments were randomized within each leaf pair and replicated three times.

Three leaf disks from each leaf were placed in the barrel of a plastic syringe and extracted for 1 hour with 99:1 methanol-HCl. The disks were rinsed twice at 20-minute intervals with 5 ml portions of the extracting solution. The concentration of anthocyanin was determined spectrophotometrically at 525 nm. The results were reported as absorbance/3 leaf disks.

#### EXPERIMENT 2: EVALUATION OF UV DOSE - RESPONSE FOR COLEUS

In an effort to further define the UV dose-response relationships in coleus, 4-week-old coleus cuttings were exposed to broad-band UV radiation produced by two FS-40 lamps, with appropriate plastic filters. The filters, 5 mil Mylar, 5 mil CA, 10 mil CA, and 20 mil CA were used. The spectral cutoff characteristics of these filters are illustrated in Fig. 7.

Plants were grown on a 16 hour photoperiod at ca 28/25° day/night temperature. PAR was provided by four 1500 ma cool white fluorescent lamps. Plants were exposed to UV irradiation during the last 6 hours of the photoperiod for 3, 6, and 8 days at UV irradiances and UV levels shown in Table 1. UV measurements were taken from 250-369 nm with an Optronics Laboratories Model 725 UV-B spectroradiometer (4,6).

After 3, 6, and 8 days of treatment, the second set of leaves below the apex was extracted with methanol-HCl (99:1). The anthocyanin concentration in each leaf was measured spectrophotometrically at 525 nm and reported as absorbance/gm fresh weight. Plants were scored for visual leaf injury based on leaf color, shape and size.



### EXPERIMENT 3. DETERMINATION OF SPECTRAL CHANGES OF METHANOL-EXTRACTABLE SUBSTANCES IN COLEUS LEAVES.

Following exposure to unfiltered and filtered UV radiation, coleus leaves were extracted with methanol-HCl (99:1). The resulting solution was concentrated by flash evaporation to less than 5 ml and diluted to 10 ml with the extracting solution. UV and visible absorption spectra were determined with a Perkin-Elmer UV-Vis spectrophotometer.

In order to classify the methanol-extractable constituents of coleus leaves, other samples were extracted with methanol, concentrated by flash evaporation, and chromatographed on paper (5, 10). Following development with butanol:acetic acid:water (BAW, 4:1:5, top layer) the chromatographs were viewed under a UV lamp. Fluorescent spots were eluted with methanol-HCl (99:1) and diluted to 2 ml. The UV and visible spectra were determined as indicated above.

### RESULTS AND DISCUSSION

#### EXPERIMENT 1: EFFECT OF UV EXPOSURE ON ANTHOCYANIN PIGMENT OF COLEUS LEAVES

The exposure of coleus to unfiltered UV radiation resulted in epidermal damage, glazing and reduction in the anthocyanin pigment content. This damage was primarily confined to the upper leaf surfaces and initially involved only those tissues exposed to the radiation. The effect of shading is shown in Fig. 1. The extractable anthocyanin of coleus leaves exposed to continuous UV radiation (1 FS-40 + 3 cool white fluorescent lamps at 16 cm) for 1-24 hours decreased with increasing time of UV exposure (Table 2). The trend in pigment reduction was most striking between 16 and 24 hours. However, due to considerable variability in initial pigment intensity, the differences were not statistically significant at the 5% level by the F test. When improved sampling techniques were used,



the anthocyanin concentration was found to be significantly decreased by exposure to unfiltered UV radiation for 12 hours (Table 3).

#### EXPERIMENT 2: EVALUATION OF UV DOSE - RESPONSE STUDIES

The leaf injury in coleus, produced by exposure to UV radiation, can be easily observed (7,8). In previous experiments with unfiltered UV radiation (a 700-900% increase in BUUV) exposure for 12 hours produced a rapid disappearance of the anthocyanin pigment without concomitant changes in leaf shape. However, exposure to FS-40 lamps filtered with 5 mil CA (42 hours under a 300-500% increase in BUUV) caused distortion of leaf margins (Figs. 2, 3). The expanding apical leaves assumed a "sickle shape," resulting from decreased development of the leaf half nearest to the UV source. Although this symptom did not preclude further leaf expansion, the curvature remained until senescence, even with a subsequent reduction in UV exposure. Both symptoms were used in developing a leaf injury index (Table 4). Using these parameters, injury produced by the UV treatments over an 8-day period ranged from slight injury, with the 10 mil CA filter, to intense injury with the 5 mil CA filter. The range of UV injury from filtered treatments is shown in Fig. 4.

The spectral transmission curves (Fig. 7) indicated that 5 mil Mylar had an effective cutoff at approximately 310-315 nm; the spectral cutoff for the CA filters ranged from approximately 286-292, depending upon the thickness of the filter.

The contrast in UV injury between the plants grown under Mylar (Fig. 5) and those grown under 5 mil CA (Fig. 6) was striking. Symptoms of UV injury under 5 mil CA included marginal distortion, glazing of the surfaces and pigment changes in the apical leaves; under 5 mil Mylar, no visible changes were observed.





Statistical analysis (Table 5) indicated that the anthocyanin content of plants irradiated under 5 mil CA decreased significantly with increases in the UV dose.

### EXPERIMENT 3: DETERMINATION OF SPECTRAL CHANGES OF METHANOL-EXTRACTABLE SUBSTANCES IN COLEUS LEAVES

The visible spectra of methanol-extractable constituents of coleus leaves were modified by exposure to UV radiation. The extracts from unirradiated control plants and those filtered with Mylar, produced strong absorbance peaks in the 412-434, 520-535, and the 640-660 nm regions. UV treatment was characterized by a reduction in the absorbance at the 520-535 peak and increases at 412-434 and 640-660 nm. The contrast between the unirradiated control plants and those irradiated for 24 hours under unfiltered UV is shown in Fig. 7.

Similar results occurred for a 6-hour unfiltered treatment when the plants were more highly pigmented. The visible spectrum for greenhouse grown coleus is shown in Fig. 8. Strong absorbance bands occurred at 420 and 525 nm, with a minor peak at 657 nm. The spectrum for the 6 hour unfiltered UV treatment was characterized by reduced absorbance at 525 nm and substantial increases at 425 and 657 nm (Fig. 9). This pattern can be used to demonstrate the effect of filtered treatments over time shown in Experiment 3. Exposures of plants to 3, 6, and 8 days of UV under FS-40 lamps + 5 mil CA were associated with progressive increases in the absorbance at 425 and 657 nm and relative decreases at 525 nm (Fig. 10).

The UV spectrum of the extracts from control plants shows absorbance peaks at 275-295 and 320-340 nm. Twenty-four hour exposure to unfiltered UV radiation reduced the absorbance in both regions (Fig. 11). The effect of UV radiation can be visualized by calculation of absorbance ratios. Table 6 contains these data for a 6-hour unfiltered treatment and the corresponding unirradiated control.



Paper chromatography and subsequent spectroscopy were used to determine the methanol-extractable components of coleus leaves responsible for the observed spectral characteristics. A summary of the spectral characteristics of these bands is given in Table 7.

Band 7 produced a large, but poorly defined peak in the region of a 210-230 nm and sharp maxima at 415 and 657 nm. UV treatments increased the absorbance at 415 and 657 nm (Figs. 12 and 13). Band 6 contributed a small peak at 290 nm and a large peak at 330 nm, which decreased with intensity of UV treatment (Figs. 14 and 15). Band 4 (Figs. 16 and 17) absorbed at 270 and 330 nm and appeared as a large UV-absorbing area on the chromatograph. The spectrum of band 2 showed strong peaks at 280 and 520-535 nm (Figs. 18 and 19). UV treatment was particularly effective in eliminating this band.

With the exception of band 2, which appeared to be a derivative of cyanidin (11), identification of the compounds involved in this separation has not been made.

The destructive effect of UV radiation on coleus leaf constituents which absorb in this region was expected. However phenols, such as cinnamic or ferulic acids, did not increase measurably due to UV treatment.

#### SUMMARY

Short-term exposure (12 to 16 hours) of 3-week-old coleus plants to high levels of UV radiation (an increase in BUV of 100% or more) produced by unfiltered FS-40 fluorescent sunlamps resulted in a significant decrease in the concentration of anthocyanin that could be extracted from the leaves. Although the concentration of the pigment in unexposed plants was variable and depended on other environmental conditions, such as temperature, light intensity, and nutrition of the plants, the specific decrease due to UV treatment was observed by evaluation of the changes which occur in portions



of the same leaf and in the opposite leaves of the same plant. Significant degradation of the anthocyanin pigment occurred after 12 hours of exposure and was intensified with an increase in exposure up to 24 hours. Complete degradation of the pigment occurred within 36 to 48 hours, depending on the experimental material. The quantitative relationship between the concentration of the pigment and the UV dose, observed at a high level of exposure (700 to 900% increase in BUUV), did not apply proportionally to a lower level of UV radiation (less than a 100% increase in BUUV) produced with CA filters. Exposure to a 100 percent increase in BUUV caused distortion of the leaf margins, and an inhibition of leaf expansion, as well as some degradation of anthocyanin.

The visual leaf injury index appears to be a more useful indicator of injury than does the measured anthocyanin content of coleus leaves at levels of exposure constituting less than a 100 percent increase in BUUV. However, the anthocyanin content determined after a 24-hour UV exposure to a 300 to 500 percent increase in BUUV was more reliable as an indicator of UV dosage than was the index. The effect of UV radiation on the methanol-extractable constituents of coleus leaves was particularly dramatic. The UV-absorbing components of the leaves were substantially decreased by UV exposure. In the visible range, the strong absorbance at 525 (anthocyanin) was decreased by maximum UV exposure, whereas the peaks at 410-420 and 657 were increased. Semeniuk (7) reported large increases in absorption of extracts from coleus leaves examined at 268, 286, and 328 nm. This relationship was not resolved by this study. The reason for increased absorbance at 410-420 and 657 nm following UV exposure was not determined.



Although the underlying mechanisms of UV injury in coleus remain to be explained, the plant responses to enhanced UV radiation observed (glazing of leaf surfaces, deformation of expanding leaves and specific decreases in anthocyanin pigmentation) may serve as useful predictors of UV damage in higher plant.





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Table 1. Weighted and unweighted UV measurements<sup>1</sup> of UV irradiance under FS-40 fluorescent sunlamps covered with various filters.

Filter	UV-B Irradiance $\text{mWm}^{-2}$	BUV <sup>2</sup> $\text{mWm}^{-2}$
5 mil Mylar	129.7	0.23
20 mil CA	982.0	4.52
10 mil CA	1494.5	9.44
5 mil CA	2161.4	18.15

<sup>1</sup>Measurements were taken with a spectroradiometer 12.7 cm above the canopy

<sup>2</sup>A base line level of biologically effective UV (BUV) radiation was equivalent to  $3.06 \text{ mWm}^{-2}$  for a standard Beltsville sun.



Table 2. Concentration of anthocyanin in Coleus blumei Benth. leaves as a function of UV exposure and node position. Basal node = 1. (Absorbance/g fresh wt. of leaf tissue). One unfiltered FS-40 lamp placed 16 cm above the top of the plants.

Time of Exposure (Hours)	Average anthocyanin concentration (Abs/g fresh wt.) 525 nm					
	-UV			+UV		
	Node 4	Node 5	Average	Node 4	Node 5	Average
1	.499	.501	.500	.560	.509	.535
2	.352	.470	.411	.384	.403	.394
4	.298	.330	.314	.370	.405	.388
8	.600	.386	.493	.575	.696	.636
16	.385	.445	.415	.394	.292	.343
24	.451	.518	.485	.083	.007	.045





Table 3. Change in anthocyanin concentration of Coleus blumei Benth. leaves with duration of UV exposure under one unfiltered FS-40 lamp at 30 cm. (Absorbance per 3-leaf disks, each 11 mm diameter.)

Time of Exposure	Anthocyanin	Concentration	Average
	Node 5	Abs/3 leaf disks Node 4	
0	.506a*	.492a	.499
12	.264 b	.269b	.267
24	.117 c	.215 b	.166

\*Mean followed by a common letter are not significantly different at the 5% level according to Duncan's multiple range test.



Table 4. Effect of UV exposure on visual leaf injury index.

Duration of Exposure*	Injury index			
	Mylar	5 mil CA	10 mil CA	20 mil CA
Days*				
3	0	+	-	0
6	0	++	-	0
8	0	+++	+	0
Weighted mWm <sup>2</sup>	0.23	18.15	9.44	4.52
% Increase in BUUV	-	493	209	48

\* 6 hours/day

Key: 0 = no injury  
 + = moderate (slight leaf distortion with negligible pigment loss)  
 ++ = severe (leaf distortion with 50% pigment loss)  
 +++ = intense (leaf distortion with 50% pigment loss)



Table 5. Anthocyanin concentration in Coleus blumei Benth. leaves as a function of UV exposure.

Duration of Exposure**	Anthocyanin Concentration (Abs/g fresh weight)			
Days	Mylar	5 mil CA	10 mil CA	20 mil CA
3	1.66 ab**	1.50 ab	1.27 bcde	1.74 a
6	1.28 bcde	1.04 cde	1.51 ab	1.43 abc
8	1.37 abcd	0.84 e	1.49 abc	1.47 ab
Weighted $mWm^{-2}$	0.23	18.15	9.44	4.52
% Increase in BUUV	-	493	209	48

\* 6 hours/day

\*\*Means followed by a common letter are not significantly different at the 5% level according to Duncan's multiple range test.



Table 6. Absorbance/g fresh weight for leaf extracts from unfiltered UV treatments (FS-40 at 30 cm).

Treatment	Absorbance/gm at Wavelength (nm)			Absorbance Ratio		
	330	420	525	320/420	420/525	330/525
Control	0.472	0.052	0.068	9.07	0.77	6.94
Unfiltered UV	0.351	0.100	0.14	3.51	7.14	25.0





Table 7. Chromatographic separation of methanol-extractable components from the leaves of Coleus blumei Benth.

Band	Rf (x 100)	Color		Absorption maxima (nm)	
		Visible	UV	Visible	UV
1	10.5-19.2	Pink	Rose	*	*
2	24.5-33.3	Red	Rose	520-535	280
3	35.1	x	x	*	*
4	36.0-57.9	x	dark brown (absorbing)	*	270, 330
5	59.6	x	x	*	*
6	61.4-94.7	x	bright blue	*	280, 330
7	94.7	Yellow-green	yellow-brown	415, 657	210, 230

\* No observed maximum

x Colorless



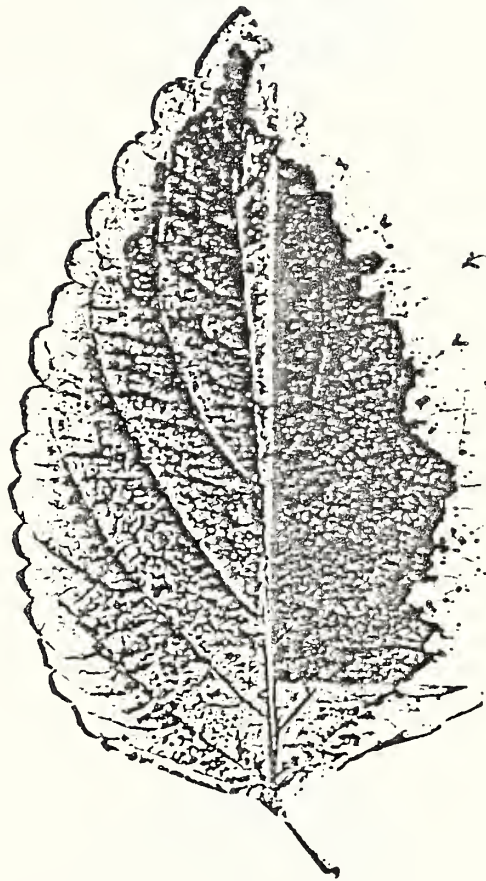
## LEGENDS TO FIGURES

- Figure 1. Influence of shading Coleus blumei Benth. leaf from UV radiation.
- Figure 2. Abnormal growth of upper Coleus blumei Benth. leaves exposed to UV-B (2 FS-40 lamps filtered with 5 mil cellulose acetate and mounted at 30 cm). Left to right: Plants irradiated 6 hour/day for 6, 9, and 12 days, respectively.
- Figure 3. Response of expanded leaves of Coleus blumei Benth. to UV-B. Left to right: Plants irradiated for 6, 9, and 12 days respectively under 2 FS-40 fluorescent sunlamps filtered with 5 mil cellulose acetate lamps mounted 30 cm above the plants.
- Figure 4. Effect of UV radiation on 4-week-old Coleus blumei Benth cuttings irradiated 6 hour/day for 8 days. Filter treatments (l-r) 5 mil Mylar, 5 mil CA, 10 mil CA, and 20 mil CA.
- Figure 5. Four-week-old Coleus blumei Benth. cutting irradiated 6 hour/day for 8 days under 2 FS-40 lamps filtered with 5 mil Mylar.
- Figure 6. Four-week-old Coleus blumei Benth. cutting irradiated 6 hour/day for 8 days under 2 FS-40 lamps filtered with 5 mil CA at a 493% increase in BUV.
- Figure 7. UV spectral irradiance obtained under 2 FS-40 fluorescent sunlamps filtered with 5 mil CA (setup 1), 10 mil CA (setup 2), 20 mil CA (setup 3) and 5 mil Mylar (setup 4) at a distance of cm above the canopy.
- Figure 8. Visible absorption spectrum of methanol-extractable constituents of Coleus blumei Benth. leaves. Plants were grown in a greenhouse without supplemental UV treatment.
- Figure 9. Visible absorption spectrum of methanol-extractable constituents of Coleus blumei Benth. leaves after 6 hours of exposure to unfiltered FS-40 fluorescent sunlamps.



- Figure 10. Visible absorption spectra of methanol-extractable constituents of Coleus blumei Benth. leaves irradiated 6 hour/day for 3, 6, and 8 days under FS-40 lamps filtered with 5 mil CA.
- Figure 11. Comparative UV absorption spectra of methanol-extractable components of Coleus blumei Benth. leaves left unirradiated (bottom curve) or exposed to unfiltered FS-40 fluorescent sunlamps for 6 hours (top curve).
- Figure 12. Visible absorption spectrum of band 7 obtained from extract of Coleus blumei Benth. leaves following 6 hours of UV irradiation under unfiltered FS-40 lamps.
- Figure 13. Visible absorption spectrum of band 7 obtained from extract of Coleus blumei Benth. leaves taken from an unirradiated control plant.
- Figure 14. UV absorption spectrum of band 6 obtained from extract of Coleus blumei Benth. leaves taken from unirradiated control plant.
- Figure 15. UV absorption spectrum of band 6 obtained from extract of Coleus blumei Benth. leaves exposed for 6 days to 2 FS-40 lamps + 5 mil CA at 30 cm above the canopy.
- Figure 16. UV absorption spectrum of band 4 obtained from extract of Coleus blumei Benth. leaves taken from unirradiated control plant.
- Figure 17. UV absorption spectrum of band 4 obtained from extract of Coleus blumei Benth. leaves following 6 hour of UV irradiation under unfiltered FS-40 lamps.
- Figure 18. Visible absorption spectrum of band 2 obtained from extract of Coleus blumei Benth. leaves taken from unirradiated control plant.
- Figure 19. UV absorption spectrum of band 2 obtained from extract of Coleus blumei Benth leaves taken from unirradiated control plants.





SHADED AREA

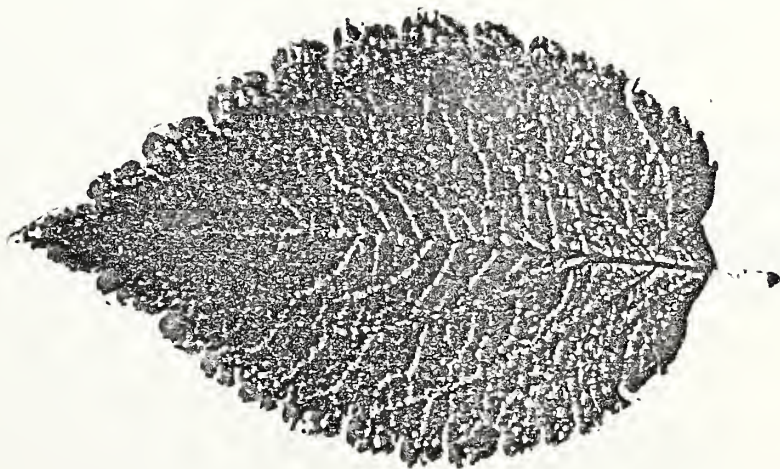
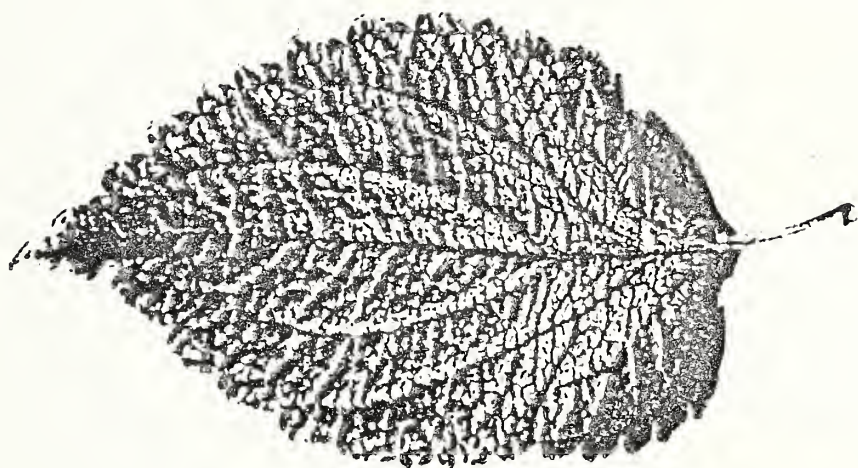
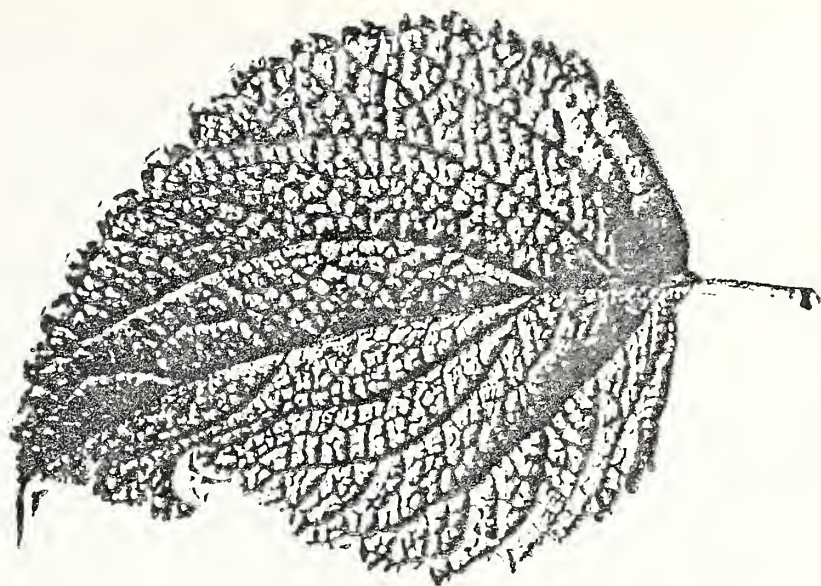




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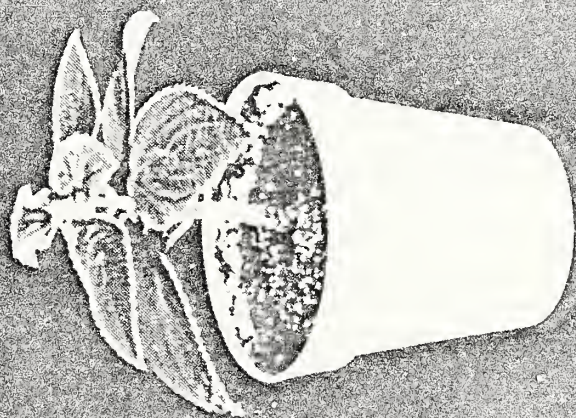
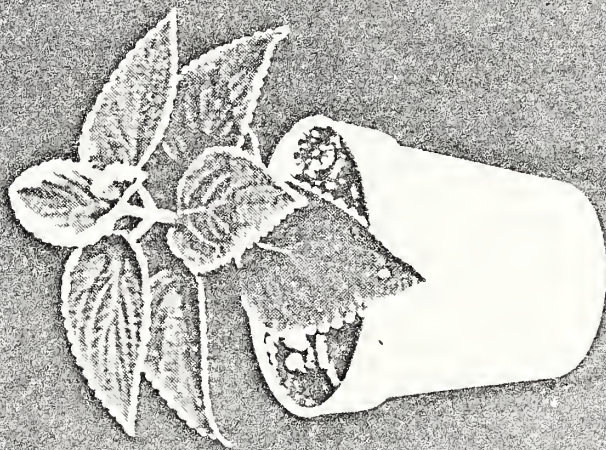
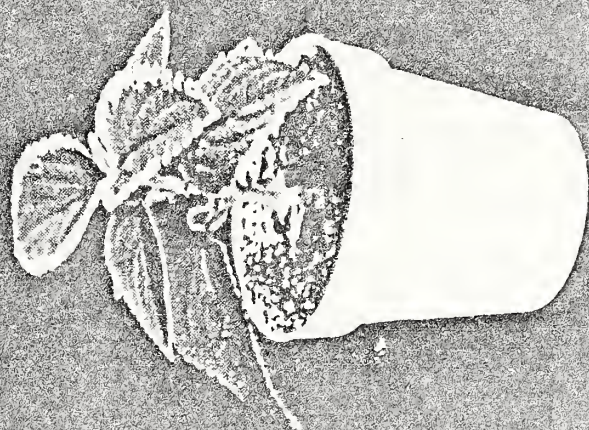








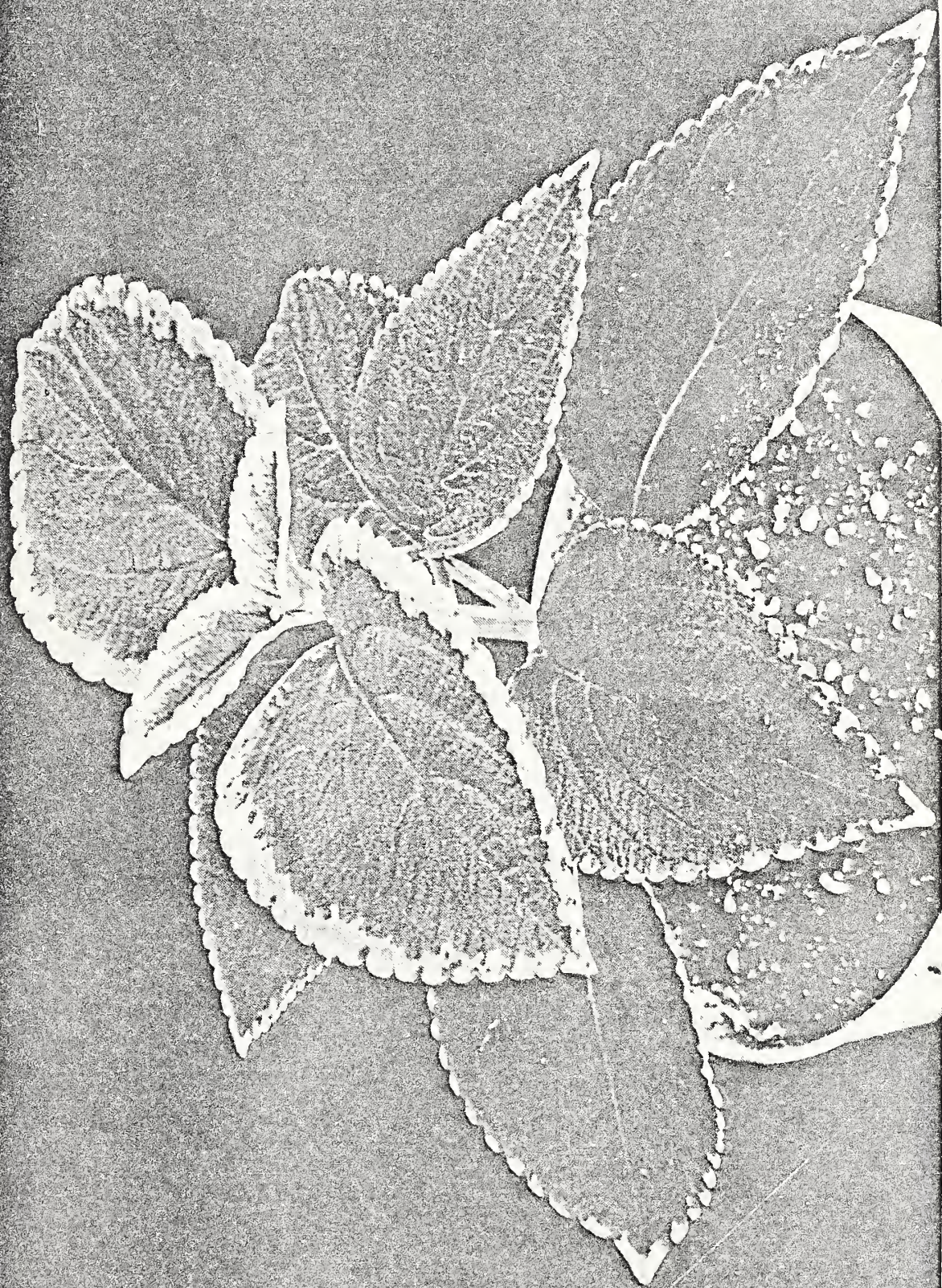
















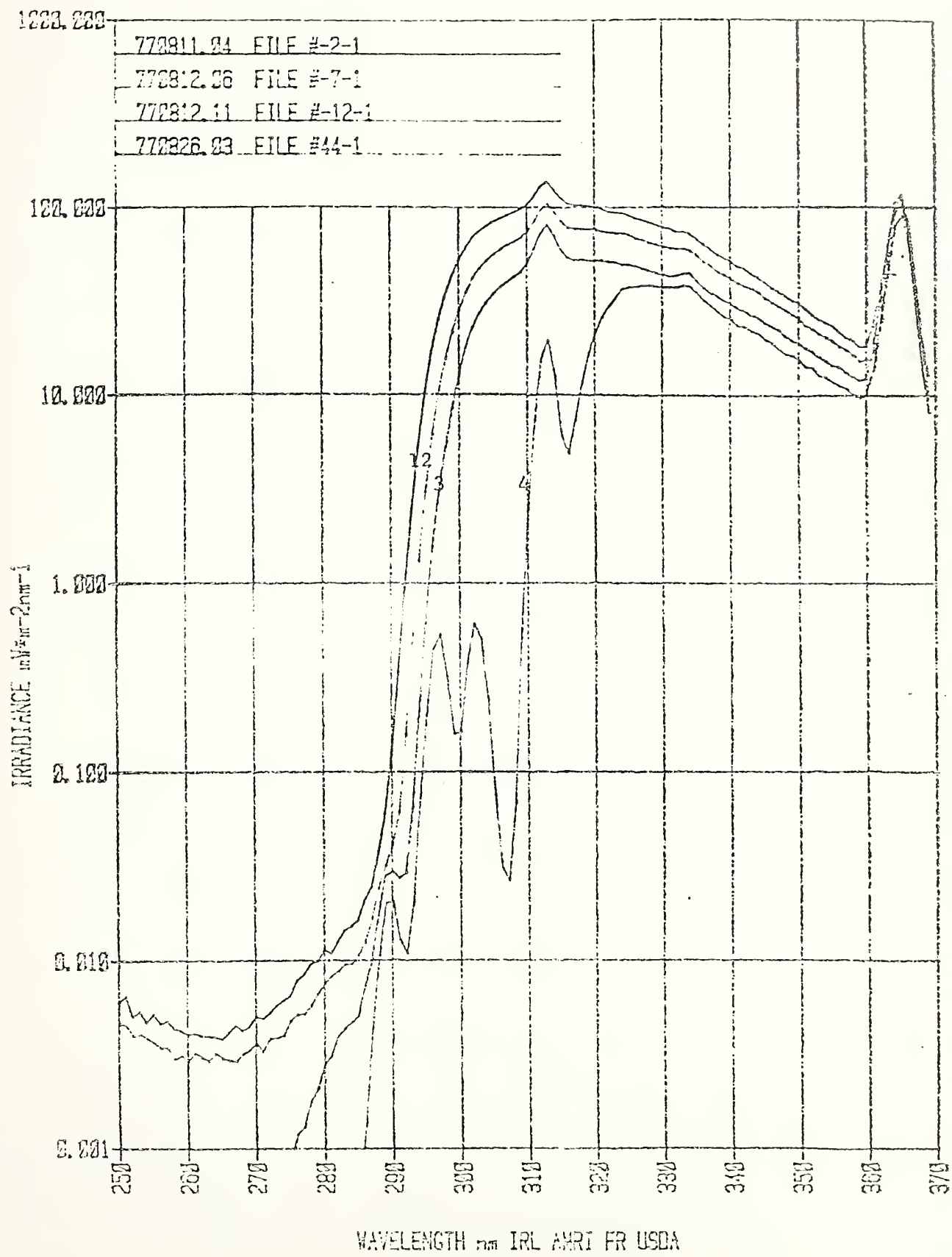








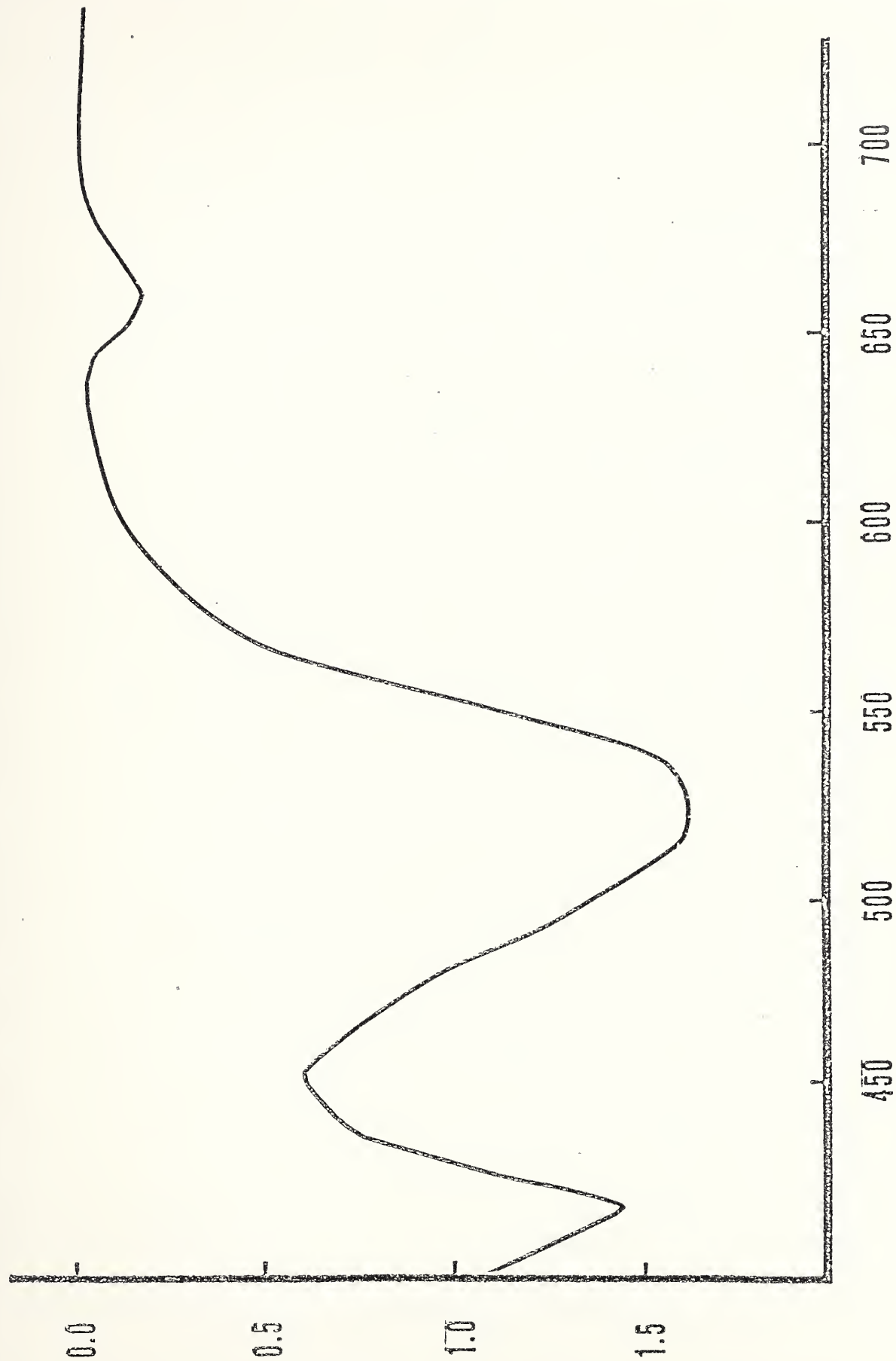
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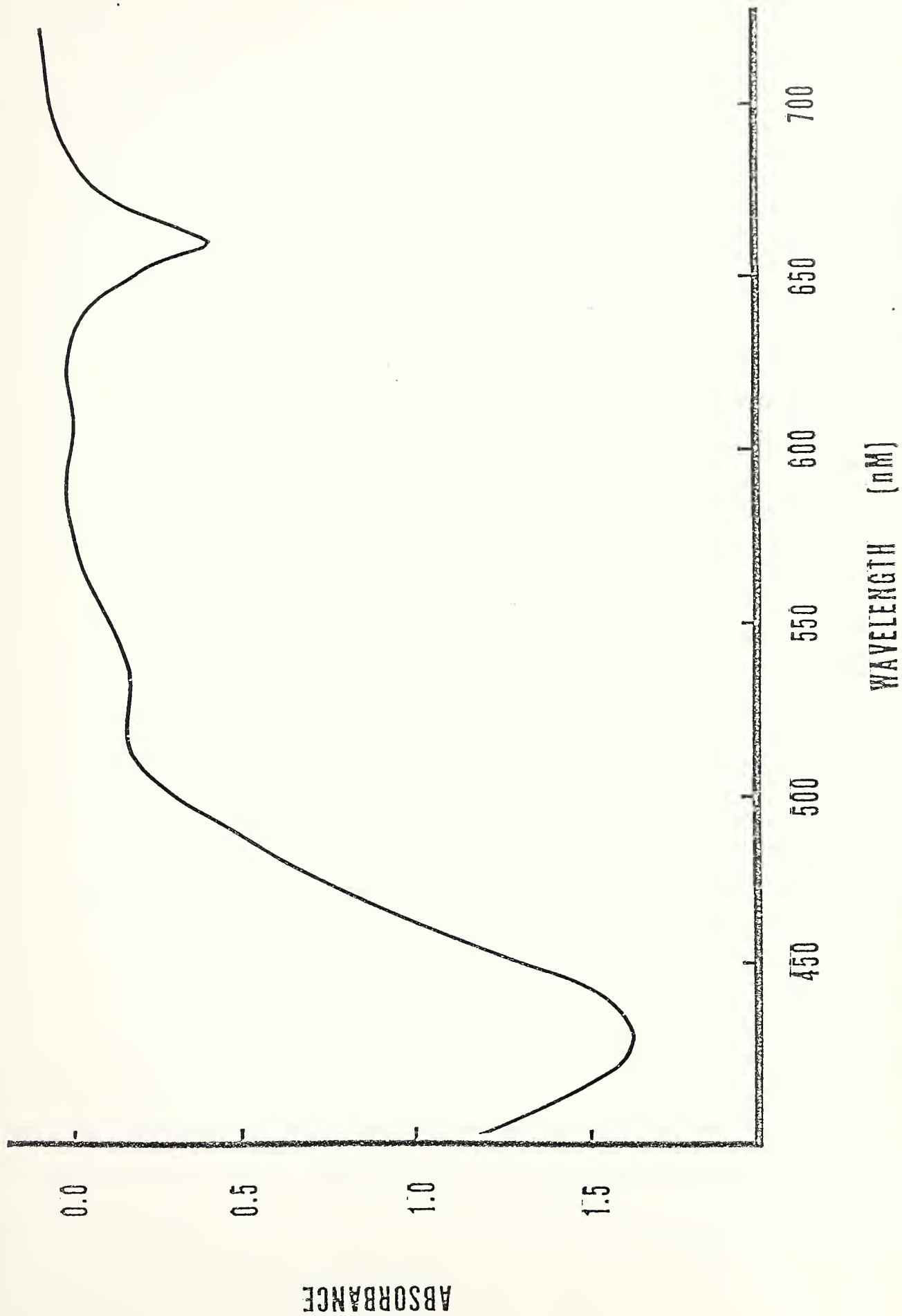


ABSORBANCE

WAVELENGTH (nm)

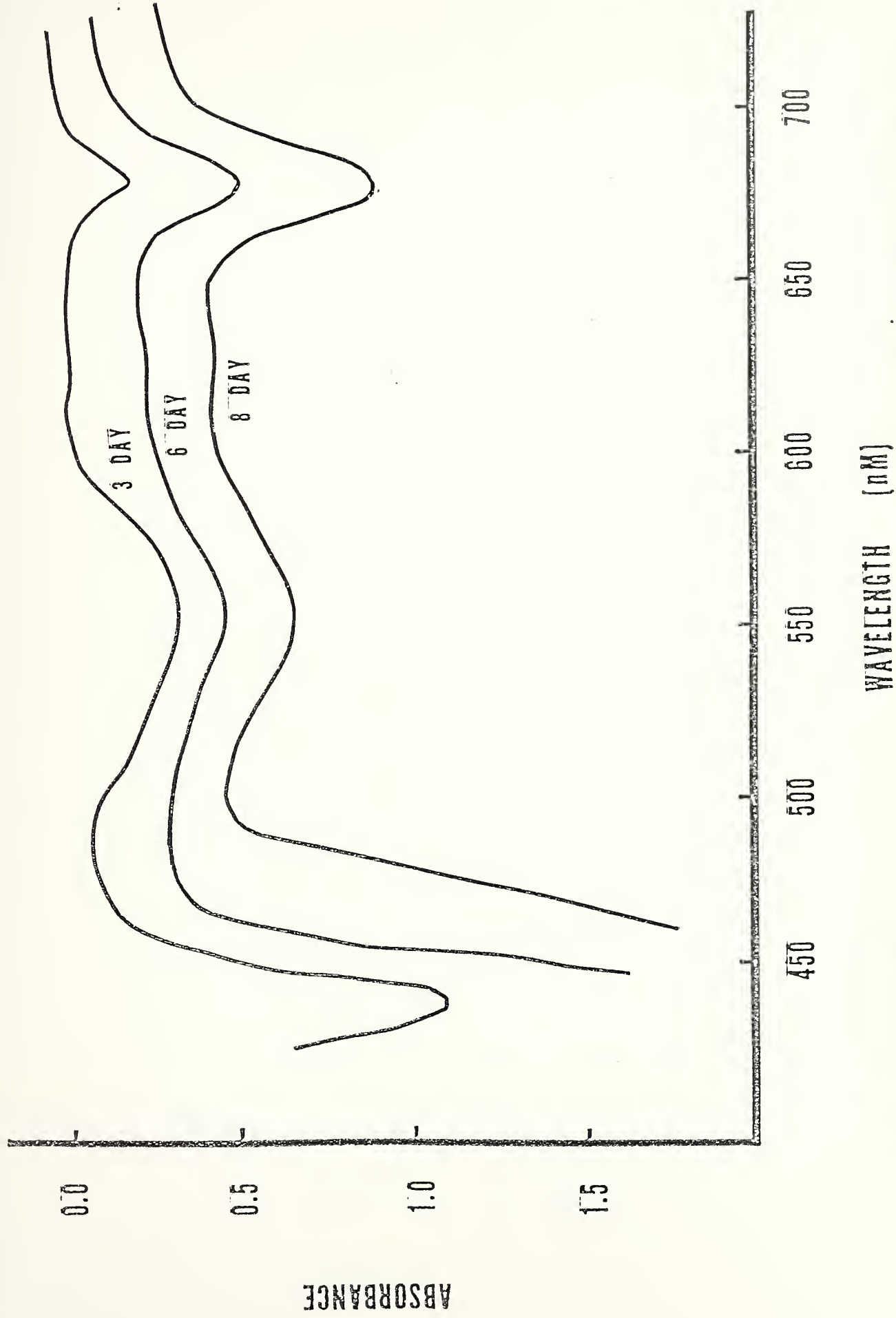




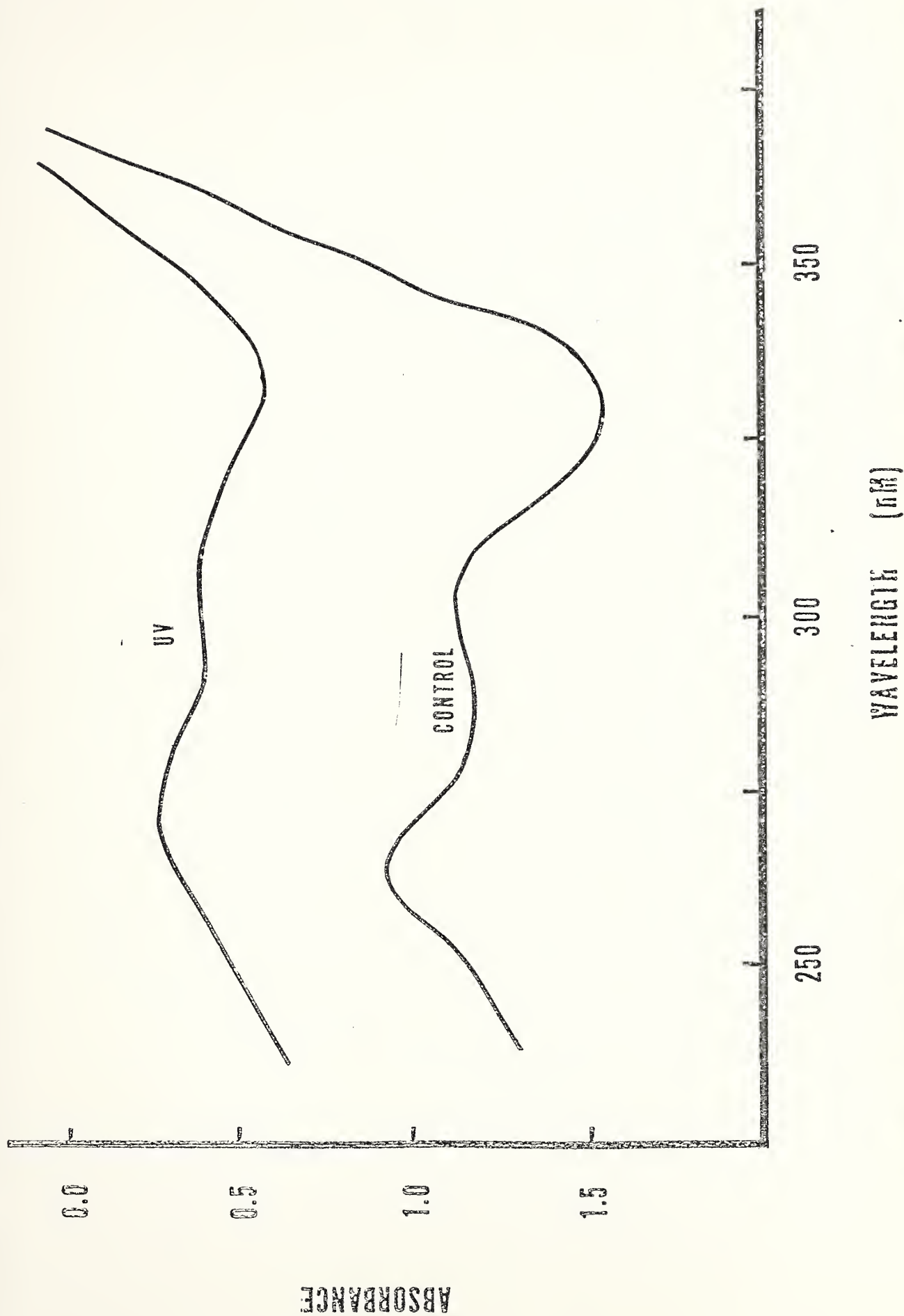




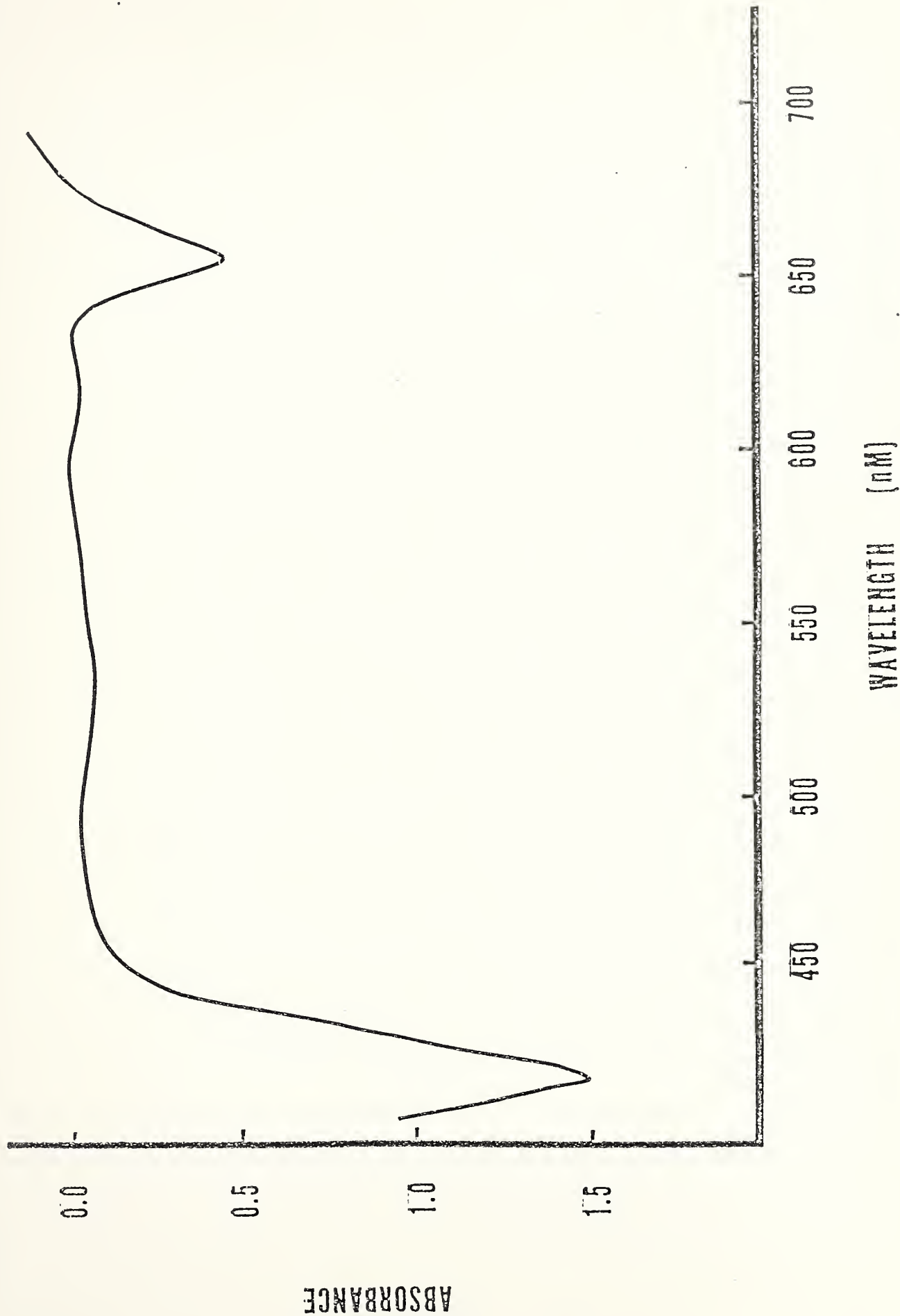




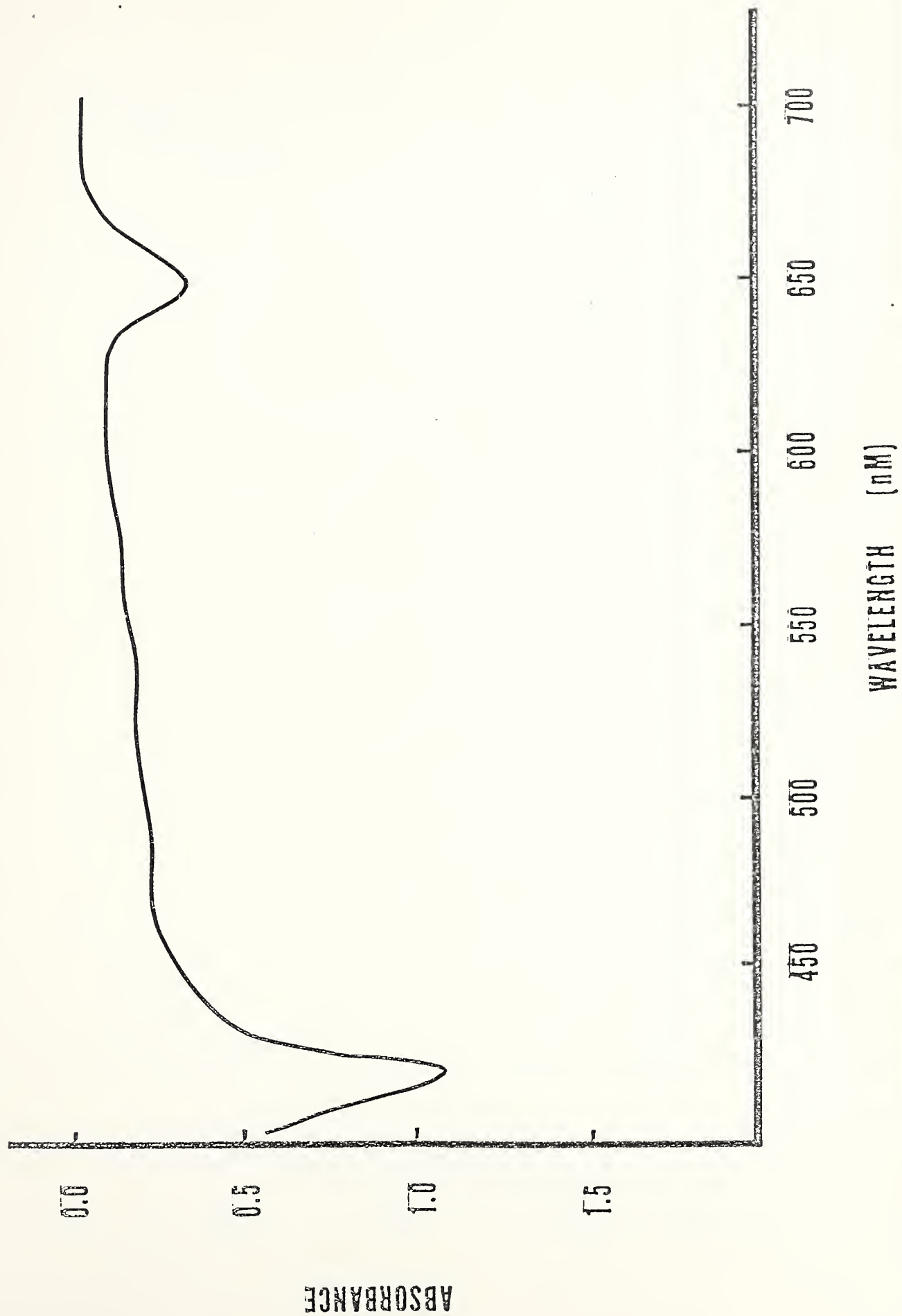






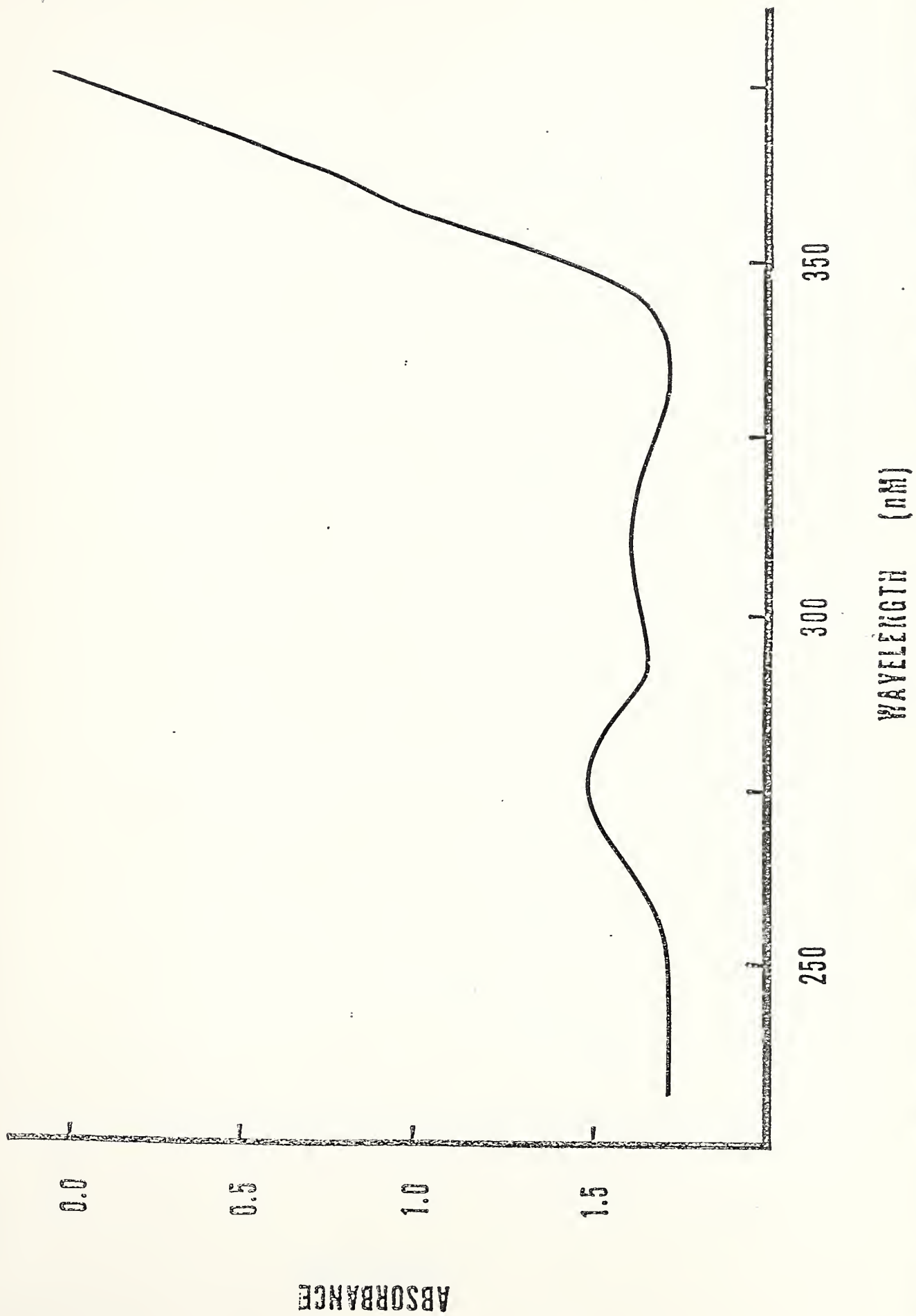














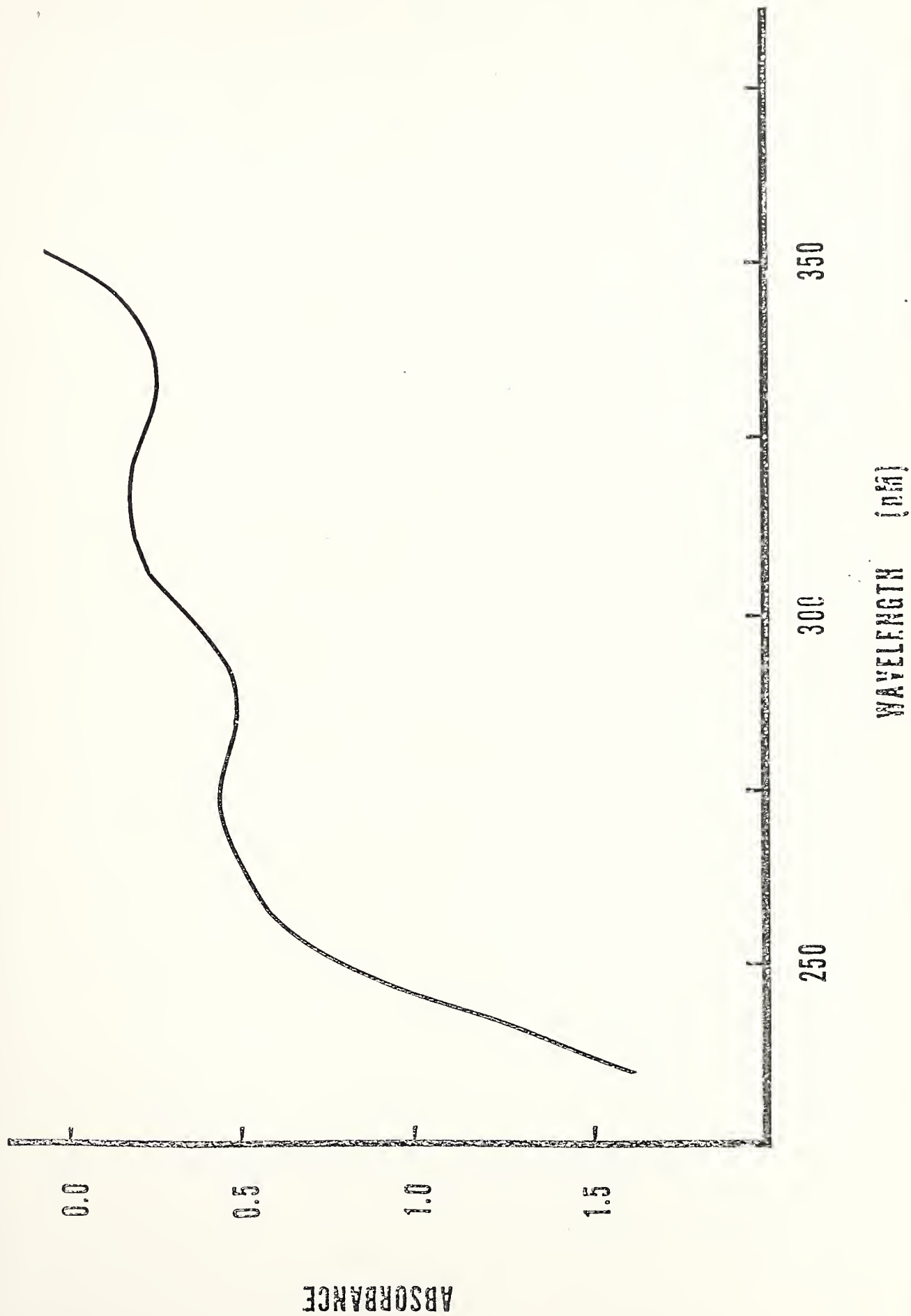
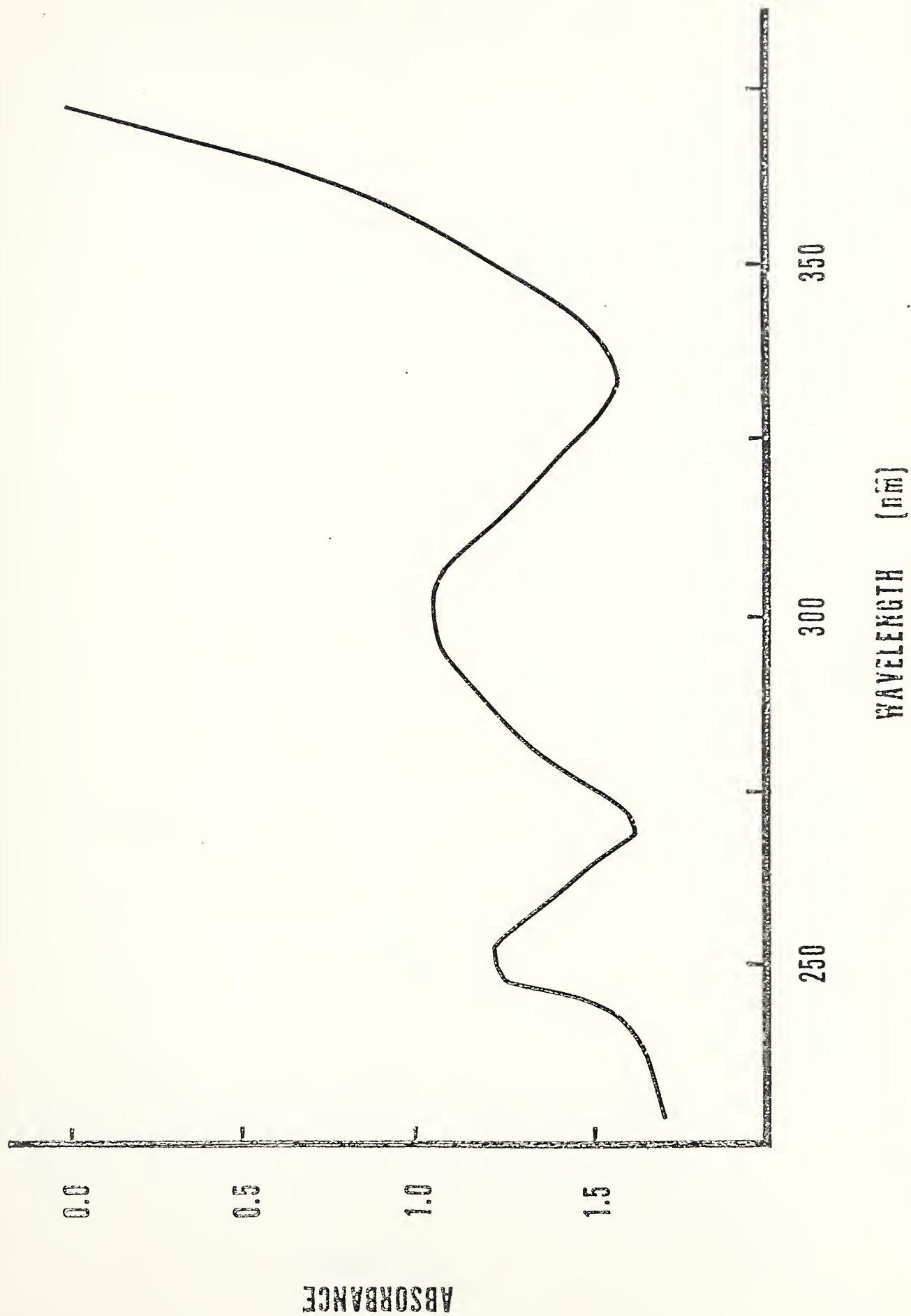
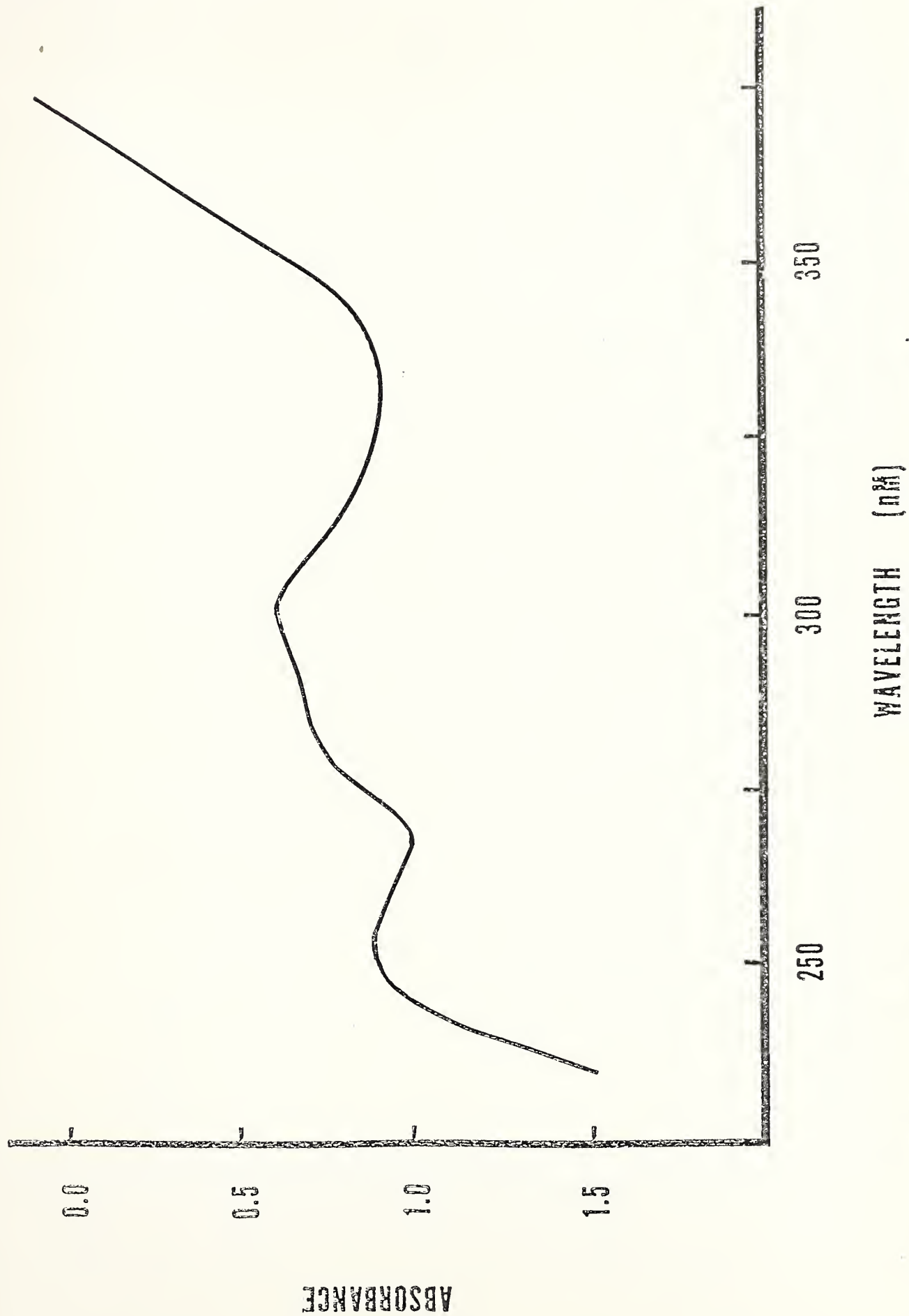




Fig-10

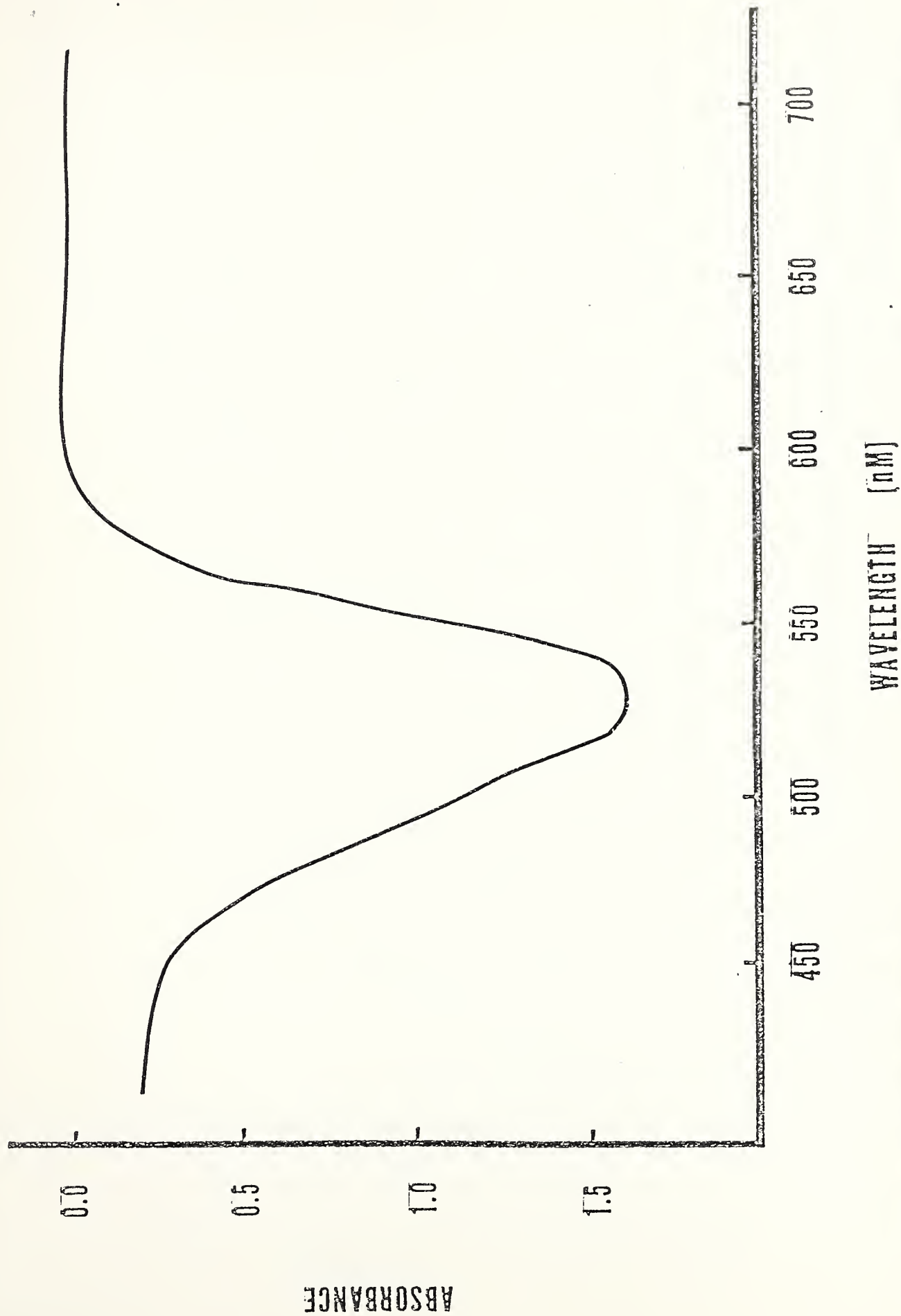




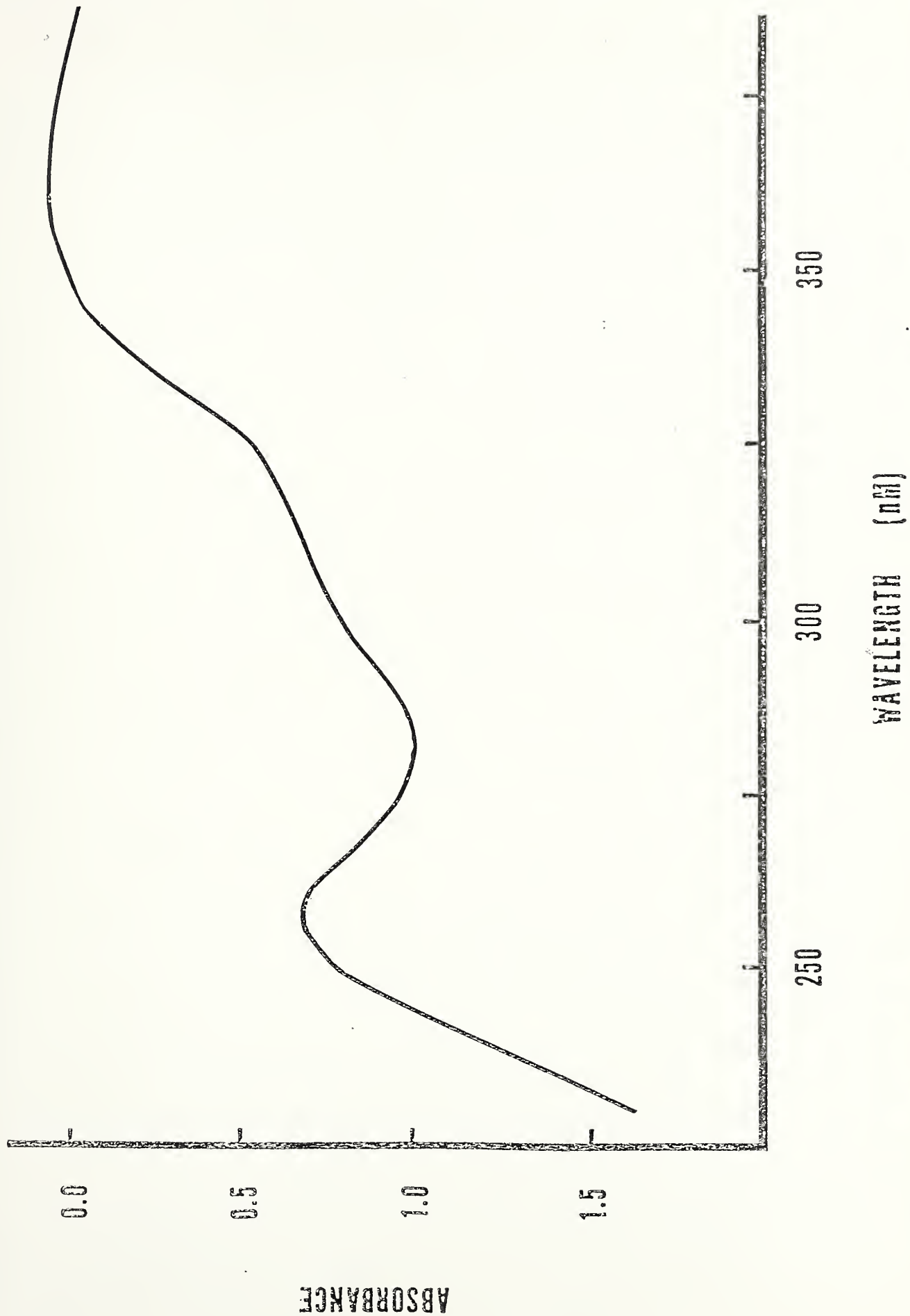


















FINAL REPORT

EFFECTS OF UV-B RADIATION ON PHOTOSYNTHESIS AND GROWTH  
OF SELECTED AGRICULTURAL CROPS

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## ABSTRACT

Selected snap bean, soybean, clover, cotton, cucumber and wheat varieties were exposed to UV-B radiation over 2 to 6 week periods (6 or 24 hr/day) under greenhouse and growth chamber conditions. Biologically effective UV-B irradiances based on an experimentally determined action spectrum ranged from 1-8 Sun Equivalents (SE). Carbon dioxide exchange rates (CER), plant biomass production, stomatal diffusion and transpiration were determined. UV-B effects on CER and foliar diffusivities were correlated with the amount of visible injury induced (i.e., chlorosis, leaf and petiole pigmentation, leaf stipple). In the absence of visible injury, CER, leaf conductances, and biomass production were not measurably depressed in experimental plants given the extended UV-B exposures.

The plant species differed markedly in their susceptibilities to UV-B radiation. Greenhouse-grown snap beans and soybeans sustained high levels before they were injured (in excess of 125 hr exposures--3 weeks, 6 hr/day--to more than 4 SE). Wheat and clover were not injured by the maximum UV-B exposures tested (3-4 week treatments at 2 SE). *Poinsett* cucumber developed marginal chlorosis when irradiated for 1-3 weeks at 1-2 Sun Equivalents. Cotton petioles and midveins at the leaf bases became (red) pigmented in the 4 SE trials. Snap bean plants grown under low-light conditions in the growth chamber were more sensitive to UV-B injury than when grown in the greenhouse.



## INTRODUCTION

Ultraviolet B (280-320 nm) irradiation corresponding to enhanced levels reaching the earth's surface due to projected stratospheric ozone ( $O_3$ ) destruction by halocarbon emissions has been reported to suppress photosynthesis in certain agricultural plant species (1-4). Some data indicate that vegetation grown and exposed to UV-B under low photo-synthetically active radiation (400-700 nm) regimes may be more sensitive than when irradiated in bright sunlight (4). Photorepair mechanisms may be important in mitigating the plant damage.

Experimental plants showing UV-B depressed carbon dioxide exchange rates (CER) produced less plant biomass when irradiated over an extended period of time. Brandle et al. (1) observed chloroplast structural damage in UV-B injured leaves and correlated it with reduced Photosystem II activity. This was proposed as a causal factor in depressing photosynthesis and growth. They further concluded from their studies that CER suppression was not caused by stomatal closure induced by UV-B damage to the leaf epidermis. Stomatal resistance to gas exchange might increase without specific injury to the epidermal cells, however, as mesophyll and chloroplast disruption can result in stomata closure as a consequence of higher  $CO_2$  concentrations within the leaves (as well as other factors).

Little information has been published concerning visible injury on UV-B irradiated leaves showing reduced photosynthesis and growth. It was not possible to effectively assess the extent to which growth reduction correlated with tissue damage and to integrate this with CER suppression.

Considering the potential importance of the reports referred to above on impending governmental regulatory actions dealing with the environmental



impact of chlorofluorocarbon use in the United States, this research was conducted as part of a Federal interagency cooperative project to corroborate earlier findings and to expand the available information on UV-B radiation effects on agricultural plants. Research reported here present data on UV-B exposures and the exposure ranges required to measurably depress CO<sub>2</sub> assimilation in selected crop plants. Special attention was given to integrating the results with incipient visible injury. UV-B effects on gas diffusion through the upper and lower leaf surfaces under typical growth chamber and greenhouse conditions were also investigated.

Soybean, cotton, wheat, clover, cucumber, and two snap bean varieties were tested.

#### METHODS AND MATERIALS

Experimental plants, with the exception of cucumber, were grown and irradiated with UV-B under (i) common growth chamber conditions or (ii) in a glass greenhouse. The plants were cultured in 15-cm dia. clay pots containing a sand-silt (1:3) soil mix. They were fertilized weekly with Peter's<sup>1/</sup> fertilizer containing micronutrients. The (heated or wet-pad, fan cooled) greenhouse was equipped with a high-volume charcoal air filtration system to prevent plant injury from oxidant air pollution (5). Cucumbers were grown and irradiated in fiberglass greenhouses in 12.5-cm dia. pots filled with a peat-vermiculite mix (1:1). They were watered daily with 1/4 strength Hoagland's solution during the first 3 weeks and with 1/2 strength Hoagland's solution thereafter.

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<sup>1/</sup> Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.



The cucumber plants tested were part of another U.S. Department of Agriculture (USDA) study (6) concerned with UV-B injury and growth reduction in the sensitive *Poinsett* variety and more tolerant *Ashley* variety.

Growth chamber (Controlled Environments, Inc., Model PCW 36) conditions are shown in Figure 1. The plants were given 12-hr photo-periods (0800-2000) utilizing cool white fluorescent + incandescent lighting. Greenhouse plants received solar radiation. Photosynthetically Active Radiation (PAR) was monitored with a LI-COR 190-S Quantum Sensor.

UV-B exposures were carried out using standardized procedures (7) developed for USDA UV-B studies employing FS-40 sunlamps with 5 mil cellulose acetate (CA) filters. Mylar filters were used for the controls. Spectral irradiances (250-370 nm range) were measured with an Optronic Laboratories, Inc., Model 725 spectroradiometer developed by the Instruments Research Laboratory (IRL), SEA, USDA (8). Spectroradiometer data were obtained for every nanometer over this range. Routine broadband UV-B measurements were made with an IRL UV-B Radiometer. Plants were irradiated for 6 hours per day (between 1000-1600) or, in some companion experiments, for 24 hours per day.

Figure 2 shows a typical UV-B spectral irradiance curve for the CA-filtered FS-40 sunlamp system obtained at a distance providing a total irradiance for the 280-320 nm wavelength range of  $1079 \text{ mWm}^{-2}$ . The plant injury Action Spectrum, empirically determined for cucumber and certain other plants (9), and the weighted biologically effective ultraviolet irradiance (BUV) are also plotted. For the FS-40 sunlamp system the summed BUV over the 280-320 nm region, called the Action Integral  $\Sigma_A$ , represented approximately 1% of the total UV-B irradiance





(cf.  $10 \text{ mWm}^{-2}$  in Figure 2). This varies somewhat as the CA transmission changes with exposure time (solarizes). UV-B spectral transmission of the CA filters was routinely monitored with a Beckman DB UV-visible recording spectrophotometer. Spectroradiometer data were also obtained for different exposure times. The CA filters were changed every 4 days.

The unweighted and BUV-weighted irradiance curves in Figure 2 represent UV-B exposures for Figure 1 experiments.

Paired (control vs UV-irradiated) intact leaves or whole plants stratified according to age, position, stage of development, and condition, were used in each CER trial. The paired foliar subjects were examined simultaneously under identical conditions in matched Physiological Activity and Diagnostic Chambers (PhAcDC) which permitted  $\text{CO}_2$  and water vapor exchange rates and leaf and air temperatures to be continuously monitored during the experimental runs (10,11). The PhAcDC tests regulate and standardize physical parameters that enter into the leaf energy balance equation [ie., Radiant Energy (input) = Reradiation + Convection + Evapotranspiration + Metabolic Energy (net photosynthesis or respiration )]. PhAcDC cuvettes were equipped with internal mini-systems for humidity and wind control. PAR was derived from Quartzline lamps filtered through a 10-cm  $\text{H}_2\text{O}$  heat filter. Dual PhAcDC experiments were conducted at PAR intensities ( $900 \pm 100 \mu\text{E m}^{-2}\text{sec}^{-1}$ ) that gave maximum, but light-unstressed, steady-state apparent photosynthesis ( $P_{\text{max}}$ ) rates. Chamber temperature, relative humidity, and wind conditions were:  $27 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%$  and  $0.5 \pm 0.1 \text{ m sec}^{-1}$ , respectively. Soil temperatures were  $25 \pm 2^\circ\text{C}$ .

Leaf, air, and soil temperatures were monitored with an 11-channel YSI Telethermometer (Probe types: T2600, T2631). Leaf temperature



calibrations and spot checking were made with a Mikron 15 IR noncontact Thermometer. Wind speeds were determined with Hastings RF-1 and AB-27 Air Meters (Probes: N-7B and S-27). Soil moisture and pH were determined with a Bouyoucos Soil Moisture Meter and the Kelway Soil pH/Moisture Tester, Model HB-2.

Each paired run required approximately 4 hours in the dual-PhAcDC systems for complete examination and diagnosis including steady-state  $P_{\max}$  rates, dark respiration rates, and leaf diffusive resistances. Relative CER data given in Tables 1 and 2 were calculated from steady-state  $P_{\max}$  rates. Foliar diffusive resistances were computed from evapotranspiration rates and data taken with a Lambda Diffusion Porometer (10,11). Plant biomass data were determined from leaf area measurements and fresh and dry weights. The experiments were conducted during January-September 1977.

## RESULTS AND DISCUSSION

### Leaf diffusive resistance: Effects of UV-B and experimental conditions

Results of experiments to investigate the influence of exposure conditions on gas diffusion through the stomata of UV-B irradiated plants are shown in Figure 1. Two snap bean varieties (Phaseolus vulgaris cvs. *Bush Blue Lake 290* and *Astro*) grown and exposed to UV-B radiation in the growth chamber and greenhouse are compared. An inset gives their relative plant biomasses at harvest.

Ratios for the upper  $r_u$  vs lower  $r_l$  leaf surface diffusive resistances to transpired water vapor are plotted as functions of the total leaf resistance  $R$ . Foliar diffusive resistances reflect the leaf health and stress physiology--responding to the moisture balance, phytotoxic agents, aging, mesophyll  $CO_2$  levels, and a number of other factors. The adaxial and abaxial surfaces were compared since it was postulated that UV-B



irradiation could cause more epidermal injury to the upper exposed surface leading to an early effect on gas permeation through this surface. Leaf diffusion was more restricted in growth chamber plants than greenhouse-grown plants. [Growth chamber plants:  $R = 3-10 \text{ sec cm}^{-1}$ ; greenhouse plants;  $R = 1-3 \text{ sec cm}^{-1}$ .] Stomatal opening and development, light dependent processes, were undoubtedly suppressed under the lower PAR levels ( $270 \mu\text{E m}^{-2} \text{ sec}^{-1}$ ) of the growth chamber. Consequently, relative photosynthetic rates would be restricted by diffusion limitations as well as by the lower PAR available for light-harvesting chloroplast reactions. PhAcDC studies indicated that snap bean leaves required PAR intensities of about  $800 \mu\text{E m}^{-2} \text{ sec}^{-1}$  for maximum photosynthesis.

Diffusive resistances given in Fig. 1 are mean values for all (<3/4 expanded) first, second and third trifoliates sampled biweekly over the 4-week UV-B irradiation period. The data were taken during mid-morning to noon--on sunny days in the greenhouse--on well-watered plants. Lower surfaces of greenhouse and chamber-grown leaves exhibiting moderate diffusive resistances (i.e.,  $2-5 \text{ sec cm}^{-1}$ ) were about twice as permeable as the upper surfaces for both UV-B exposed and control plants. The  $r_u/r_l$  ratios increased rapidly as higher or lower total diffusive resistances were measured. The arrayed data do not indicate that diffusion through the upper surfaces of UV-B irradiated plants was significantly altered relative to that of the lower surface; though, there may be a slight tendency for increased  $r_u/r_l$  ratios in UV-B exposed plants.

After several weeks in the chamber some UV-B injury was observed on *Bush Blue Lake 290* (BBL 290) bean leaves. [UV-B injury symptoms: Red pigmentation of the petioles and leaves with slight leaf stipple, i.e., scattered small "flecks" of necrotic cells.] Two to three weeks after



losing their cotyledons *BBL 290*, which is more sensitive to a number of known environmental stresses than *Astro* (10,11), showed a gradual loss of vigor in both UV-B exposed and control plants. As the foliage became stressed with time in the chamber (perhaps by inadequate photosynthesis), leaf diffusive resistances increased accordingly. The *Astro* variety withstood the growth chamber conditions better than *BBL 290* and was also less sensitive to UV-B injury. The plants were removed after 4 weeks in the growth chamber and returned to the greenhouse to check for recovery. At harvest (2 weeks later), UV irradiated *BBL 290* plants had less biomass than the controls (Fig. 1 inset). *Astro* plants exposed to UV-B radiation in the chamber did not differ statistically from the controls.

The healthy, vigorous greenhouse plants grew larger than the chamber plants (cf: C/G, Fig. 1 inset). Foliar resistances remained low throughout the experimental period. At harvest neither *BBL 290* nor *Astro* plants grown and irradiated with UV-B in the greenhouse differed in biomass from their Mylar controls. No visible UV-B injury occurred on the greenhouse plants.

The results tend to corroborate previous reports that plants under low PAR regimes may be injured more by UV-B irradiation than plants in bright light. The more sensitive *BBL 290* snap bean variety, furthermore, grew less vigorously in the low PAR chamber environment than the *Astro* cultivar. Preliminary trials with *Pennscott* clover showed this clover variety to grow well in the growth chamber. The chamber-tolerant clover was not injured by comparable BUV exposures ( $\Sigma A = 10 \text{ mWm}^{-2}$ , 6 hrs/day) during a 4-week trial.

#### UV-B effects on CER, plant injury, and growth

Table I summarizes the results of CER experiments conducted during the spring and summer of 1977 on six crop plants exposed to increasing







UV-B doses. Table 2 gives an abridged array showing: visible injury index ratings for the PhAcDC investigated leaves; comparative leaf conductances for evapotranspired water vapor (See footnote, Table 2); and relative plant biomasses of the irradiated plants given as percent of control. The experimental plants were cultured and irradiated with specified UV-B doses in the greenhouse and transferred with their paired controls to leaf or whole plant dual-PhAcDC systems for examination and characterization. The CER trials were restricted to UV-B exposures that produced no more than 20% leaf injury (Injury Index II). Data for snap bean, soybean, cotton, cucumber, and clover were taken on intact leaves or trifoliates. Wheat and additional clover data were obtained from whole plant studies. Equivalent numbers of leaves on the controls and UV-B exposed plants were used. The data were normalized on a weight basis.

UV-B exposed leaves showing no visible injury at the time of testing did not statistically differ from their paired controls in CER or foliar conductances, nor was plant biomass reduced by the extended exposures. CER values were lowered roughly in proportion to the amount of visibly damaged leaf tissue. Snap bean leaves with an Injury Index I rating (1-10% injury) showed mean CER values 8% below those of the controls. Soybean and cucumber leaves assigned to the Injury Index II class (10-20% injury) gave CER values averaging 14% and 16% below the controls.

High UV-B radiation levels were required to injure greenhouse grown snap bean and soybean plants--in excess of 125 hrs exposure (3 weeks, 6 hrs/day) to more than  $12.5 \text{ mWm}^{-2}$  of biologically effective UV-B radiation. Cotton was marked by this dosage. *Poinsett* cucumber was the most sensitive plant tested. Wheat and clover were not injured by the highest doses given in those particular experiments. Visible symptoms of injury for cucumber



and cotton were well-defined chlorosis along the leaf margins (cucumber) and red pigmentation of the petioles and juncture with the leaf base (cotton). Snap bean injury symptoms were described previously. UV-B injured soybean leaves showed increased pigmentation or bronzing of the leaves with scattered necrotic stipple.

Dividing the  $\Sigma_A(\text{mWm}^{-2})$  in Table 1 and Figure 1 by  $3.06 \text{ mWm}^{-2}$  gives plant BUV exposures relative to one control sunshine equivalent SE. One SE is the weighted BUV integrated over the UV-B region for the control sunshine used at the Beltsville Agricultural Research Center (6,8,9). Five  $\text{mWm}^{-2}$ , the minimum  $\Sigma_A$  included in Table 1, represents 1.6 SE, or a 60% increase above 1 SE. One hundred hours' daytime exposure to 1.6 and 2.1 SE was insufficient to cause injury to any of the soil-grown greenhouse plants tested. *Gregg* cotton developed basal leaf and petiolar pigmentation in the 4 SE exposure tests, but no cellular necrosis or statistically significant reduction in CER. The very high UV-B levels required to visibly injure greenhouse snap bean and soybean plants, 8 SE, combined with nighttime irradiation (the plants were irradiated 300 hrs, 24 hrs/day) are exceptionally adverse treatments and will not be discussed further. The data are included to show the range of exposures given.

*Poinsett* cucumber leaves showed some chlorosis when subjected to ca. 1.2 SE. [See Krizek (6) for experimental data.] The sensitivity of this cucumber variety varied slightly with season, increasing in the fall and winter and decreasing in the spring and summer. The seasonal effect was attributed largely to photorepair in plants grown under the higher PAR. PhAcDC examined *Poinsett* leaves exposed to 1.6 and 2.1 SE showed mean Injury Index ratings of I and II based on the amount of marginal chlorosis



observed (cf. Table 2). Nonchlorotic portions of the leaves appeared to be healthy and functional. Predicted biomass (dry weight) loss, based on data generated from the larger population from which the plants were drawn (48 plants), indicated that 1.6 and 2.1 SE exposures would be expected to cause 3% and 6% reduction in plant dry weights, respectively (6). This was less than the mean leaf chlorosis and reduction in CER observed.

Increased BUV corresponding to 1.6 - 2.1 control sun equivalents represent values greater than the 1.4 SE predicted for the maximum stratospheric ozone depletion due to chlorofluorocarbon emissions. Experimental data generated from this study give no evidence that the snap bean, soybean, wheat, clover, or cotton varieties tested should be measurably damaged by the proposed UV-B increases. The potential exists for injury to *Poinsett* cucumber plants but anticipated growth reductions of the magnitudes expected would be difficult to statistically detect in nature even when some observable chlorotic injury might result.



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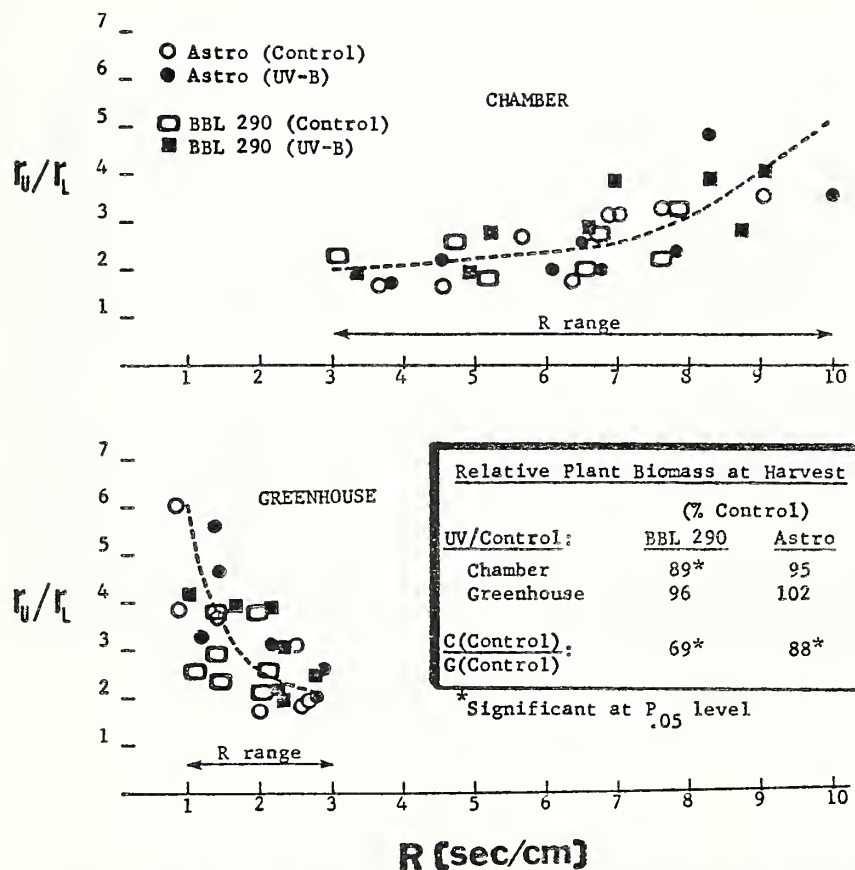
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Experimental Conditions	Growth Chamber	Greenhouse
PAR Light ( $\mu E\ m^{-2}\ sec^{-1}$ )	$270 \pm 30$	1300 (sunny day)
BUV $\Sigma_A$ ( $mW\ m^{-2}$ )	$10 \pm 2.5$	$10 \pm 2.5$
Temp ( $^{\circ}C$ ) [day/night]	[23/16]	[20-30/15-20]
R H (%)	35-55	Usual Range: 30-60
Experimental Period: Jan - Mar, 1977.		

Figure 1. Relative foliar resistances to transpired water vapor diffusion through the upper  $r_u$  and lower  $r_l$  leaf surfaces of UV-B irradiated and control plants plotted as functions of total leaf resistance  $R(sec/cm)$ . The data compare plants exposed under growth chamber and greenhouse conditions. Experimental conditions and relative plant biomass at harvest (8 weeks of age) are summarized in block inserts.





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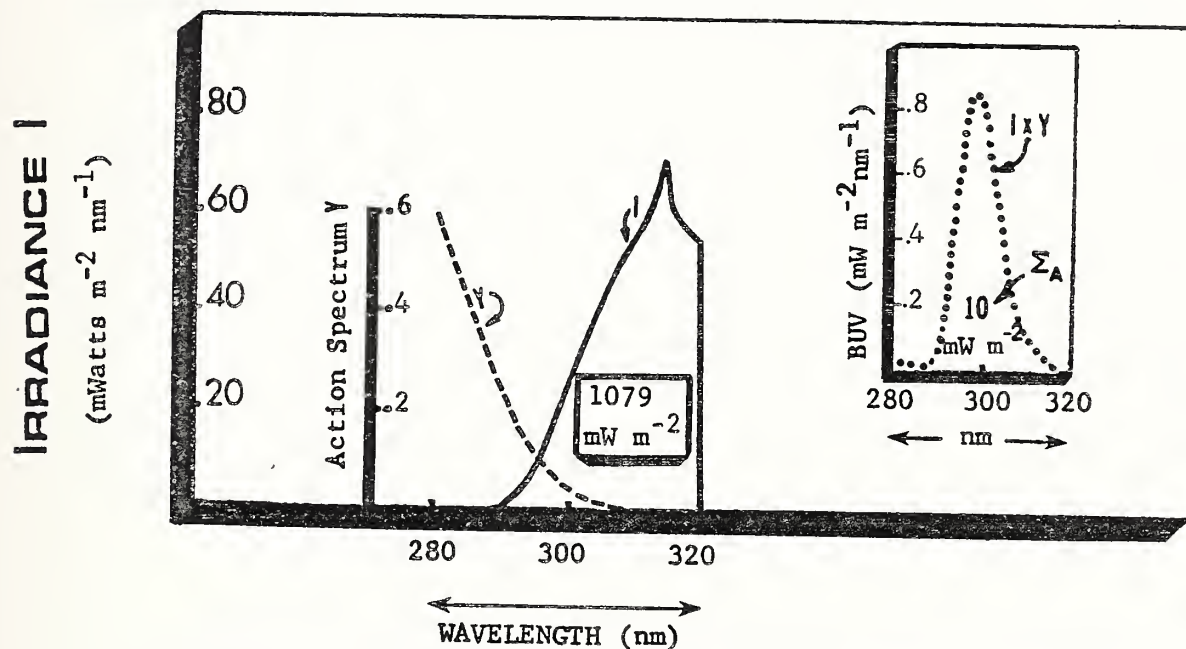


Figure 2. UV-B irradiance curve for Figure 1 experiments produced by the FS-40 sunlamp systems filtered through 5 mil cellulose acetate (exposed 6 hours). Also shown are the Action Spectrum for UV injury to cucumber (and other) plants (9), and the Biologically-harmful UV Action Integral  $\Sigma_A$  (inset). The Action Integral represents the integrated product of the Action Spectrum Y times the Irradiance I as a function of wavelength.



Table 1. Summary of relative photosynthetic rates (net carbon dioxide exchange rates, CER)<sup>a</sup> for UV-B irradiated and control plants [n = 5-8 replications per treatment].

UV-B Exposure				Relative CER					
[Expt <sup>1</sup> 1. Period: Apr.-Sept. 1977]				(% of Control)					
Exposure Range <sup>b</sup> $\Sigma A$ (mWatts m <sup>-2</sup> ) (Cf. Fig. 2)	UV-B Exposure (Hours)	Exposure Class	CER Test (Weeks)	Snap beans "BBL 290"	Soybean "York"	Clover "Pennscott"	Wheat "Monon"	Cotton "Gregg"	Cucumber "Poinsett"
5.0±0.5(6 hr/day)	100±20	A	2-3	MEANS INSIDE BOUNDARY NOT SIGNIFICANTLY DIFFERENT					
6.5±0.5( " )	"	B	3-4	105	99	103	107		84*
12.5±2.5( " )	125±25	C	4-6	97	102			95	
25±5 (24 hr/day)	300±50	D	"	92*	86*				

<sup>a</sup>CER for intact leaves calculated as  $\mu\text{g CO}_2 \text{ dm}^{-2} \text{ min}^{-1}$ ; Wheat and clover normalized on weight basis.

<sup>b</sup>One control sun equivalent (SE) equals 3.06 mWatts m<sup>-2</sup> based on action spectrum given.

\*Means Significant at P<sub>0.05</sub> level

Table 2. Summary Table showing relative net carbon dioxide exchange rates for UV-B exposed and control plants arrayed to include leaf injury indices, leaf conductance ratios for transpired water vapor, and relative plant biomass.

Plant/Variety	UV-B Exposure Class [See Table 1]	Plant Biomass (% Control)	Relative CER (% Control)	Ratio <sup>a</sup> $L_{UV}/L_C$	Leaf Injury Index <sup>b</sup>	Symptoms of Injury
Snap bean	C	105	97	1.0	0	No visible injury
"Bush Blue Lake 290"	D	94	92*	0.9	I	Leaf pigmentation; stipple
Soybean	C	96	102	1.1	0	No visible injury
"York"	D	92	86*	0.9	II	Leaf bronzing; stipple
Clover "Pennscott"	B	101	103	1.1	0	No visible injury
Wheat "Monon"	B	99	107	---	0	No visible injury
Cotton "Gregg"	C	93	95	1.0	Tr	Pigmentation of petiole and base of leaf
Cucumber	A	97 <sup>c</sup>	94	1.0	I	Marginal chlorosis of leaf
"Poinsett"	B	94 <sup>c</sup>	84*	0.9	II	" " " "

<sup>a</sup> $L_{UV}/L_C$  : Ratio of leaf conductance L (reciprocal of leaf resistance) for UV-B exposed and control plants. Conductance coefficients linearly relate gas exchange rates with concentration gradients.

<sup>b</sup>Injury Index Scale: 0 (no visible injury); Tr (<1% injury); I (1-10%); II (10-20%)

<sup>c</sup>Plant biomass (dry weight) based on regression data generated from 48 cucumber plants







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